AWARD NUMBER: W81XWH-14-2-0129

TITLE: Restoring Bladder Function by Spinal Cord Neuromodulation in SCI

PRINCIPAL INVESTIGATOR: Dr. Daniel Lu

CONTRACTING ORGANIZATION: UNIVERSITY OF CALIFORNIA, LOS ANGLELES
LOS ANGLELES, CA 90095

REPORT DATE: October 2016

TYPE OF REPORT: ANNUAL

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2016	Annual	30 Sep 2015 - 29 Sep 2016

4. TITLE AND SUBTITLE
Restoring Bladder Function by Spinal Cord Neuromodulation in SCI

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-14-2-0129

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Daniel C. Lu, M.D., Ph. D. 300 Stein Plaza. Ste 562. Los Angeles, CA. 90095

E-Mail:dclu@mednet.ucla.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
UNIVERSITY OF CALIFORNIA, LOS ANGLELES
10833 Le Conte Ave, Los Angeles, CA. 90095

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
We are submitting our Annual progress report for our grant entitled "Restoring Bladder Function by Spinal Cord Neuromodulation in SCI" (SC103209).Over the last year, we have initiated and completed the pilot study on five naïve subjects and their follow-up and started to delineate the best magnetic stimulating parameter for restoring bladder function in SCI patients. As you will see in the report, we have noticed a significant difference between high frequency and low frequency magnetic stimulations in order to restoring urinary functions in SCI individuals. This also correlates with some electrophsiological findings. In the upcoming year, we are planning on enrolling more subjects to continue testing our hypothesis and complete the pilot phase of the study. As you can appreciate from the progress report, we remain on time to accomplish the tasks set forth in the study. We believe this study will yield important information to restore bladder function in SCI patients that will make a dramatic impact on the lives of those living with SCI.

15. SUBJECT TERMS
Spinal Cord Injury, Urinary Function, Magnetic Stimulation

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	98	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

1. INTRODUCTION:

The central objective of this project is to use **non-invasive neuromodulation that can produce improved bladder function by enabling the function of spared circuitry in the spinal cord**. This normalization of the spinal cord function is accomplished through a process of functional neuroplasticity whereby neuromodulation (e.g. electromagnetic stimulation) activates spinal circuits associated with micturition. It also facilitates ascending projections for improved sensation and descending projections for volitional voiding. A subset of subjects appears to experience long-lasting improvements and can void in the absence of stimulation. A total of 18 male/female, age 18+, >1 year post (C2-T8, non-conus) injury with complete but stable severe motor paralysis (ASIA A, B) and catheterization dependent for urination will be enrolled.

2. KEYWORDS:

Corticospinal tract, Dorsal Root Ganglia, Epidural Spinal Cord Stimulation, Electromyography, Machine learning, Magnetic Stimulation, Gaussian Process Optimization, Neurogenic Detruser Overactivity, Spinal Cord Evoked Potentials, Urinary Tract Infection

3. ACCOMPLISHMENTS:

In an allied area of sensorimotor rehabilitative research, we have discovered a method that is superior to transcutaneous electrical spinal cord stimulation (TESS) in the delivery of neuromodulatory stimulation to the spinal cord. We have identified magnetic stimulation, also known as transcranial magnetic stimulation (TMS) as a better method for the following reasons.

a. Better energy delivery to deep structures. The magnitude of energy penetrating tissues and reaching the cord appears to be superior that of TESS. In our separate cohort of patients, TMS demonstrated superiority to activate the spinal cord interneurons to improve motor function, with an approximate 80% superiority in the generation of hand grip force. Additionally this positive effect is observed immediately during the first session, unlike TESS that requires a prolonged training period of 3-6 months.

b. Painless Stimulation. Our preliminary studies with TESS were very promising; however the consistently high levels of energy needed to reach the cord are prohibitive due to pain in about 40% of subjects with preserved sensation. It may be that reaching nerve roots with lower energies has provided some favorable preliminary results. We hypothesize that delivery of energy to the spinal cord is necessary to activate neuronal circuitry within the spinal cord that coordinates the activity of bladder function.

c. Access. Several manufacturers have marketed magnetic stimulation devices making these devices available to patients.

d. Durable Improvements: Our preliminary research in bladder and other regions of the spinal cord indicate that the improvements in function that occur after treatment can last for up to 6 months. This obviates the need to home use and a portable device. If patients experience a decline in function, they can return for therapy to improve function.

Because of the overwhelming superiority of the TMS device compared to TESS device, we elected to use the TMS for this study. With the TMS device, we will likely accomplish the aims set forth in the project faster and more effectively.

- **What were the major goals of the project?**

Month 0-18. Task 1

Determine the optimal stimulation parameters to enable micturition in SCI subjects.

We will determine combinations of spinal cord stimulation level (vertebral body level T10-L4, +/- coccyx), stimulation frequency (1-30 Hz) twice weekly for 24 weeks. A machine learning algorithm will guide subsequent stimulation parameters (Task 3). Subjects will be evaluated at each session for urine flow and volume, and assessments of quality of life and urinary function. Formal urodynamics will be tested monthly and at the conclusion of the 6-month study. Results will be prepared for manuscript publication.

Percent Complete: 75%

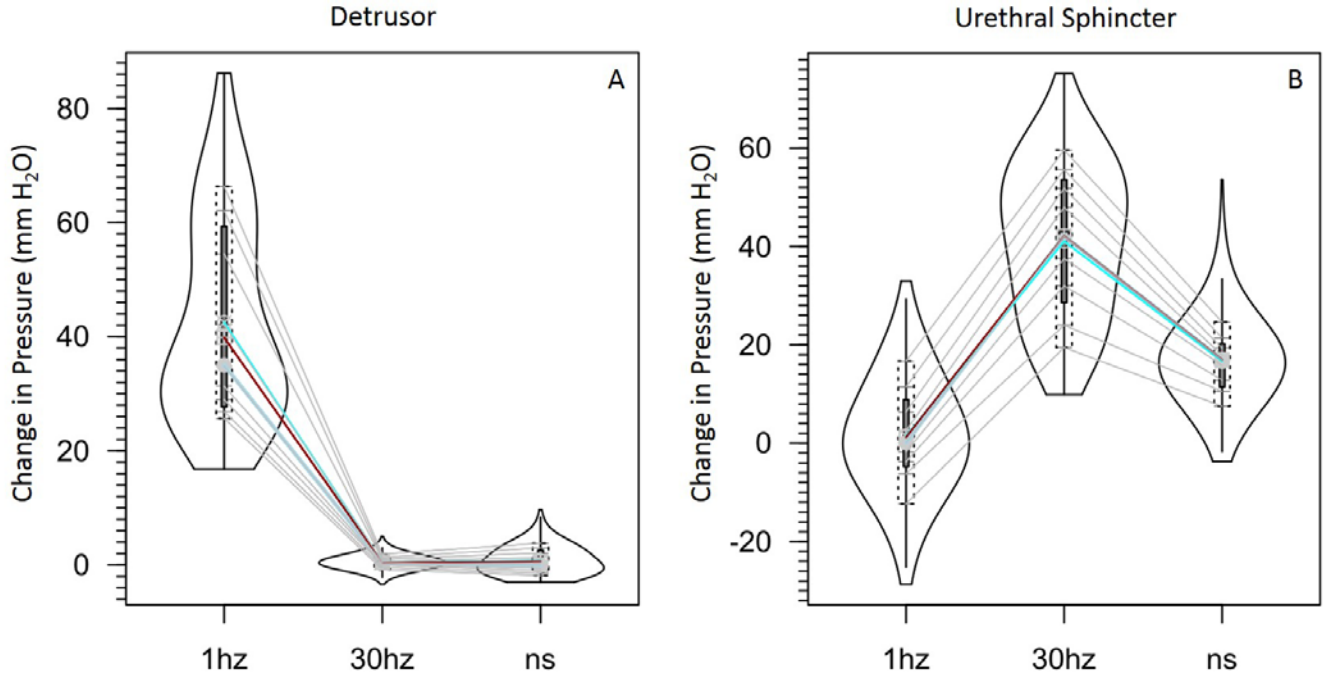


Figure 1: Violin plots of change in pressure, expressed as mm water, by stimulation condition for detrusor and urethral sphincters. Each violin shows mean and median (heavy horizontal lines) plus standard deviation (heavy vertical bars) and deciles (in light grey) using a kernal density estimator of the dtat distribution. For the measure labeled "Detrusor" (A) the 1hz condition resulted in a mean value of 42.47 (sd 17.27) while the no-stimulation and the 30hz conditions showed means of 0.47 (sd 1.06) and 0.75 (sd 2.38), respectively. For the measure labeled "Urethral sphincter" (B) the 1hz condition resulted in a mean of 1.44 (sd 10.94) while the no-stimulation and 30hz conditions showed means of 41.07 (sd 15.03) and 16.66 (sd 7.76), respectively. Analyses of variance (ANOVA)and Tukey HSD post-hoc testing were used to examine the differences between conditions in each of the two measures. In both instances, the results indicate that the 1Hz condition differed from both the no-stimulation condition and the 30Hz condition ($p < 0.0001$), but the latter two did not differ from one another.

We have several observations:

1) when there is no percutaneous magnetic stimulation, the urethral pressures increase more than the detrusor pressure increase during volitional attempt. This is indicative and a hallmark of detrusor sphincter dys-synergia (DSD), a condition that is frequently recognized in SCI subjects.

2) with low frequency stimulation, subjects have minimal change (actually mostly decrease) in urethral pressure during volitional voiding while the detrusor pressure increases significantly; hence facilitate emptying.
3) With high frequency (30Hz) stimulation, subjects have significantly increased urethral pressure with minimal to no change in detrusor pressure; hence facilitating storage.

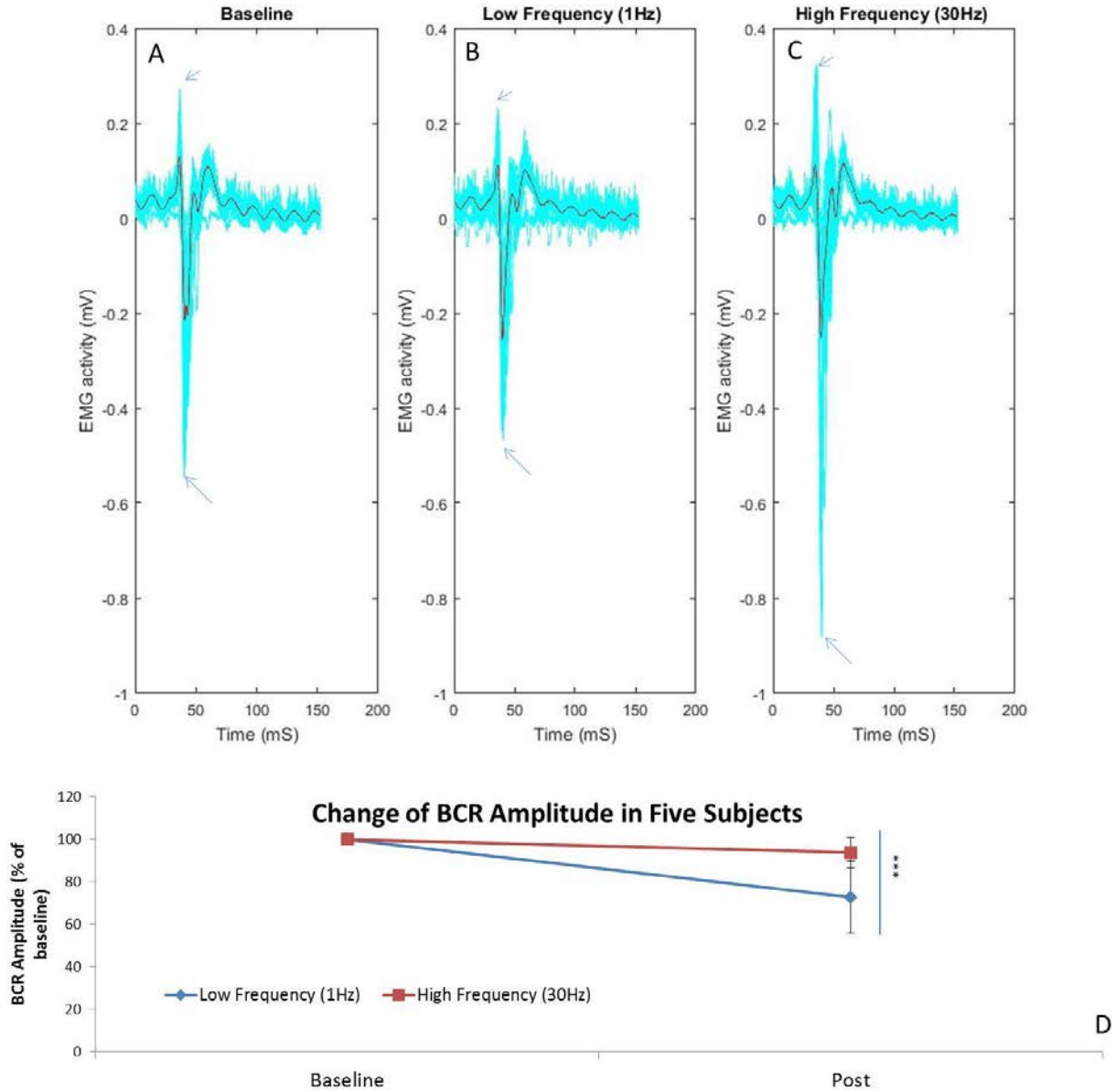


Figure 2: Change in BCR amplitude, which is measured from the perineal muscle EMG, during low frequency (1 Hz) and high frequency (30 Hz) transcutaneous magnetic spinal cord stimulation. A, B & C. An example of measured the BCR from subject C at Baseline (A), after low frequency stimulation (B) and after high frequency stimulation (C) respectively; blue = individual electrical recordings; red = average. D = amplitude changes in all five subjects. Note a significantly higher degree of reduction of BCR amplitude after low frequency stimulation when

compared to high frequency stimulation. Student's t-test: *** = $p < 0.0001$. BCR = bulbocavernosus reflex.

Several observations:

1) Low frequency transcutaneous magnetic stimulation significantly reduced the BCR amplitude in all five subjects. In contrast, high frequency stimulation resulted in either further increased amplitude or no significant change (Figure 2). The average BCR latency was 35.2 ± 5.3 ms, which is similar to the latency of the BCR in normal individuals22. The baseline amplitude, however, ranged from 490-3800 μV; amplitudes that are about 10-100 times greater than those of normal individuals22. During low frequency stimulation, the BCR amplitude was significantly decreased to between 440-3100 μV compared to the unstimulated baseline (an average reduction of 28%, $p < 0.0001$). High frequency stimulation did not alter the BCR amplitude from baseline, which ranged between 475-3700 μV ($p = 0.61$)
2) The modifications in the BCR also support the hypothesis that we accessed the micturition spinal circuitry rather than direct motor neuron stimulation as modifications of a polysynaptic reflex such as BCR require more than simple motor neuron stimulation. BCR amplitudes for our subjects at baseline were 10 to 100 times greater than those in normal individuals. This observation suggests that SCI subjects have decreased supraspinal inhibition of the BCR polysynaptic reflex. With low frequency stimulation, we observed a decrease in the BCR amplitudes and this implied an improved inhibition of the BCR polysynaptic reflex (likely via spinal micturition circuitry). However, high frequency stimulation did not decrease the BCR amplitude. (Figure 2) We hypothesize that this decrease in BCR amplitudes in chronic SCI with low frequency magnetic stimulation may signified a decreasing external sphincter motor pool hyperactivity or possibly modulating supraspinal input such that more supraspinal signal got through past the lesioned site; and hence enabling more volitional urinary functions that we observed in our subjects.

Month 19-36. Task 2
Determine the minimum training conditions to enable micturition in SCI subjects.
With the stimulation parameters from Task 1, we will determine a pre-training regimen in naïve SCI subjects that are injury matched to those in Task 1. In addition to determining the minimum number sessions, we will examine the type of training sessions, twice weekly for 24 weeks. Assessments as in Task 1. Results will be prepared for manuscript publication.
Percent Complete: 25%

Month 1-48. Task 3
Application of machine learning strategy to determining the optimal stimulation and training parameters for micturition in SCI subjects.
Data from each session will be added to a machine learning algorithm database in order to determine the most effective parameters for the most recent session and guide stimulation parameters for subsequent sessions. At the conclusion of this Task, the optimal conditions for urination will be determined and used in the clinical trial. Results will be prepared for manuscript publication.
Percent Complete: 25%

Month 37-48 Task 4
Application of the optimal stimulation and training parameters for micturition in SCI subjects in a pilot clinical trial.
This Task will combine the optimal stimulation parameters from Tasks 1-3. We will determine the optimized stimulation paradigm that can improve micturition function in naïve, SCI subjects. In this Task 12 subjects that are injury matched to Task 1-2 will be recruited. Each of 12 subjects will be tested twice weekly for 6 months. Urodynamics and self-assessments, as Task 1. Results will be prepared for manuscript publication.
Percent Complete: 10%

- **What was accomplished under these goals?**

Relevant to all above listed goals, we have completed the necessary approval to enroll subjects, recruited the necessary expertise and personnel to conduct the study with the revised device (TMS). Specifically we have (at the time of this reporting):

1. Revised and identified key personnel reflecting the change in research strategy.
2. Obtained approval for USAMRAA for device and personnel change.
3. Obtained budgetary approval of the change from USAMRAA.
4. Acquired and purchased all necessary equipment for this study.
5. Obtained UCLA IRB approval.
6. Obtained final HRPO approval.
7. Enrolled Six subjects.
8. Completed stimulation and follow-up in Five subjects

We fully anticipate to accomplish the goals set forth within the same timeline described above. In the previous 3 months of Task 1, we have begun to identify the optimal parameters for stimulation and proceeding onto the subsequent tasks as planned. Figure 1 clearly demonstrated the different effect when stimulation was applied with low and high frequencies.

- **What opportunities for training and professional development has the project provided?**
  This project has provided an opportunity for advancement in the study of molecular and cellular basis of spinal central pattern generator activity by Tianyi Niu, MD, a Neurosurgery Fellow with Dr. Lu; William Alaynick, PhD, a Project Scientist Step IV with Dr. Lu; and for the PI, Dr. Lu. A resulting manuscript has been accepted to Frontiers in Molecular Neuroscience and is attached in the Appendix. A second review manuscript with the same authors has been accepted at *Current Physical Medicine and Rehabilitation Reports* amd is attached in the Appendix. This knowledge will help to guide the further design and interpretation of this study's results. The authors are also working on another manuscript that will publish our initial encouraging results so far.

- **How were the results disseminated to communities of interest?**

  William Alaynick, PhD, Project Scientist UCLA delivered a lecture, "Spinal Central Pattern Generating Circuitry: From Bench to Bedside" at the European Neuroscience Institute at the University of Göttingen in Germany on December 16th 2014.

- **What do you plan to do during the next reporting period to accomplish the goals?**
  During the upcoming period, we plan to accomplish the following goals:

Task 1: Confirm the optimal stimulation parameters to enable micturition in chronic SCI subjects. We plan to complete the 6th subject's stimulation and then perform electrophysiologic diagnostic testing (bulbocavernosus reflex, spinal evoked potentials) in those individuals before, during and after the magnetic stimulation in order to confirm the optimal setting for stimulation. We plan to analyze the electrophysiology data to assess if we can use bulbocavernousus reflex as a more instantaneous measurement of effectiveness of magnetic stimulations. We also plan to compare some quality of life parameters for all the enrolled subjects to evaluate if there is indeed an improvement. Lastly, we plan on continue to enroll more subjects for the study.

Task 2: Determine the minimum training conditions to enable micturition in SCI subjects.
The subjects will be motor trained to prime the lumbosacral circuitry. The minimum training conditions that will enable micturition will be assessed. We have begun have begun to decipher the training conditions, while finalizing it at year 3 of the project.

Task 3: Application of machine learning strategy to determining the optimal stimulation and training parameters for micturition in SCI subjects.
Machine learning will be utilized and applied throughout in Tasks 1 and 2 to obtain the optimal conditions to enable voluntary micturition.

4. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**
  Our hypothesis that non-invasive magnetic stimulation can improve urinary bladder function has been supported in a small number of initial experiments, as planned. This warrants to continued investigation of this hypothesis and line of research.

- **What was the impact on other disciplines?**
  This research has led us to hypothesize that this type of sensorimotor rehabilitative intervention may be applicable to other indications, including central (e.g. cortical) injuries. This is only a hypothesis at this point and has not been disseminated by publication or lecture at this time.

- **What was the impact on technology transfer?**
  The PI has submitted an Invention Disclosure to the UCLA Technology Transfer Office related to the use of magnetic stimulation in rehabilitative bladder/urinary function therapy. The UCLA TTO will file a provisional patent application on behalf of the PI and UCLA.

- **What was the impact on society beyond science and technology?**
  The initial experiments are consistent with the hypotheses and objectives of this research plan and await further experimental results for confirmation.

5. **CHANGES/PROBLEMS:**

- **Changes in approach and reasons for change**

In an allied area of sensorimotor rehabilitative research, we have discovered a method that is superior to transcutaneous electrical spinal cord stimulation (TESS) in the delivery of neuromodulatory stimulation to the spinal cord. We have identified magnetic stimulation, also known as transcranial magnetic stimulation (TMS) as a better method for the following reasons.

a. Better energy delivery to deep structures. The magnitude of energy penetrating tissues and reaching the cord appears to be superior that of TESS.

b. Painless Stimulation. Our preliminary studies with TESS were very promising; however the consistently high levels of energy needed to reach the cord are prohibitive due to pain in about 40% of subjects with preserved sensation. It may be that reaching nerve roots with lower energies has provided some favorable preliminary results. We hypothesize that delivery of energy to the spinal cord is necessary to activate neuronal circuitry within the spinal cord that coordinates the activity of bladder function.

c. Access. Several manufacturers have marketed magnetic stimulation devices making these devices available to patients

d. Durable Improvements: Our preliminary research in bladder and other regions of the spinal cord indicate that the improvements in function that occur after treatment can last for up to 6 months. This obviates the need to home use and a portable device. If patients experience a decline in function, they can return for therapy to improve function.

- **Actual or anticipated problems or delays and actions or plans to resolve them**
  Nothing to Report

- **Changes that had a significant impact on expenditures**
  Nothing to Report

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
  The UCLA IRB and DoD Humans Subjects approval were modified from the use of electrical stimulation to the use of magnetic stimulation.

- **Significant changes in use or care of human subjects**
  The UCLA IRB and DoD Humans Subjects approval were modified from the use of electrical stimulation to the use of magnetic stimulation.

- **Significant changes in use or care of vertebrate animals.**
  Not Applicable

- **Significant changes in use of biohazards and/or select agents**
  Not Applicable
-

6. **PRODUCTS:**

- **Publications, conference papers, and presentations**

  - **Journal publications.**

1. Gerasimenko Y, Gorodnichev R, Puhov A, Moshonkina T, Savochin A, Selionov V, Roy RR, **Lu DC**, Edgerton VR. Initiation and modulation of locomotor circuitry output with multisite transcutaneous electrical stimulation of the spinal cord in noninjured humans. *J Neurophysiol*. 2015 Feb 1;113(3):834-42. doi: 10.1152/jn.00609.2014. Epub 2014 Nov 5
   Status of publication: Accepted
   Acknowledgment of federal support: Yes
2. Gad PN, Roy RR, Zhong H, **Lu DC**, Gerasimenko YP, Edgerton VR. Initiation of bladder voiding with epidural stimulation in paralyzed, step trained rats. *PLoS One*. 2014 Sep 29;9(9):e108184. doi: 10.1371/journal.pone.0108184. eCollection 2014.
   Status of publication: Accepted
   Acknowledgment of federal support: Yes
3. **Lu DC**, Niu T, Alaynick WA. Molecular and cellular development of spinal cord locomotor circuitry. Front Mol Neurosci. 2015 Jun 16;8:25.
   Status of publication: Accepted
   Acknowledgment of federal support: Yes
4. Niu T, Alaynick WA, **Lu DC**. Strategies and lessons in spinal cord injury rehabilitation. Current Physical Medicine and Rehabilitation Reports 2015 3;3, 206-213
   Status of publication: Accepted
   Acknowledgment of federal support: Yes

5. Hoffman H, Lee SI, Garst JH, Lu DS, Li CH, Nagasawa DT, Ghalehsari N, Jahanforouz N, Razaghy M, Espinal M, Ghavamrezaii A, Paak BH, Wu I, Sarrafzadeh M, **Lu DC**. Use of multivariate linear regression and support vector regression to predict functional

outcome after surgery for cervical spondylotic myelopathy.mJ Clin Neurosci. 2015 Sep;22(9):1444-9.
Status of publication: Accepted
Acknowledgment of federal support: Yes

6. Gerasimenko YP, **Lu DC**, Modaber M, Zdunowski S, Gad P, Sayenko DG, Morikawa E, Haakana P, Ferguson AR, Roy RR, Edgerton VR. Noninvasive Reactivation of Motor Descending Control after Paralysis. J Neurotrauma. 2015 Aug 20.
Status of publication: Accepted
Acknowledgment of federal support: Yes

7. Lee S, Mortazavi B, Hoffman H, Lu D, Li C, Paak B, Garst J, Razaghy M, Espinal M, Park E, **Lu DC**, Sarrafzadeh M. A Prediction Model for Functional Outcomes in Spinal Cord DisorderPatients using Gaussian Process Regression. IEEE J Biomed Health Inform. 2014 Nov 20
Status of publication: Accepted
Acknowledgment of federal support: Yes

- **Books or other non-periodical, one-time publications.**
  Nothing to Report

- **Other publications, conference papers, and presentations.**

1. Presentation: William Alaynick, PhD, Visiting Project Scientist UCLA delivered a lecture, "Spinal Central Pattern Generating Circuitry: From Bench to Bedside" at the European Neuroscience Institute at the University of Göttingen in Germany on December 16th 2014.

- **Website(s) or other Internet site(s)**
  Nothing to Report

- **Technologies or techniques**

In pursuit of allied research that is germane to this project we found that magnetic stimulation is more effective than electrical stimulation. We will be formally studying this discovery here and report and disseminate these results for the rehabilitative bladder/urinary function therapy.

- **Inventions, patent applications, and/or licenses**

The PI has submitted an Invention Disclosure to the UCLA Technology Transfer Office related to the use of magnetic stimulation in rehabilitative bladder/urinary function therapy. The UCLA TTO will file a provisional patent application on behalf of the PI and UCLA.

- **Other Products**
  Nothing to Report

2. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

- **What individuals have worked on the project?**
  -

Name:	Daniel C. Lu, MD, PhD
Project Role:	Principle Investigator
Researcher Identifier (e.g. ORCID ID):	1234567
Nearest person month worked:	2.52 person months per year. ~1 month this period
Contribution to Project:	Dr. Lu oversaw all aspect of research and administration of this program
Funding Support:	NIH U01
Name:	William Alaynick, PhD
Project Role:	Project Scientist
Researcher Identifier (e.g. ORCID ID):	1234567
Nearest person month worked:	2.88 person months per year. ~1 month this period
Contribution to Project:	Dr. Alaynick contributed to the IRB regulatory approval and continued intellectual development of the research plan
Funding Support:	None

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
  Nothing to Report

- **What other organizations were involved as partners?**
  Nothing to Report

3. **SPECIAL REPORTING REQUIREMENTS**

- **COLLABORATIVE AWARDS:** Not Applicable

- **QUAD CHARTS:**

Improving Cortical Sensorimotor Function and Headache with Spinal Cord Neuromodulation
Funding Opportunity: SC130209 (W81XWH-14-2-0129)
Clinical Trial Quarterly Progress Report

PI: Daniel C. Lu MD, PhD **Org:** UCLA, West LA Veterans Hospital **Award Amount:** $2,159,707

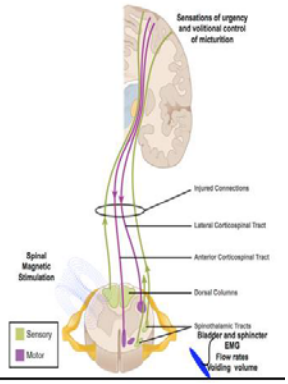
Study/Product Aim(s)

- Aim 1: Determine the optimal stimulation parameters to enable micturition in SCI subjects
- Aim 2: Determine the minimum training conditions to enable micturition in SCI subjects.
- Aim 3: Application of machine learning strategy to determining the optimal stimulation and training parameters for micturition in SCI subjects
- Aim 4: Application of the optimal stimulation and training parameters for micturition in SCI subjects in a pilot clinical trial

Approach

Aim 1 &2: Our established regimen of neuromodulation-facilitated sensorimotor rehabilitation will be applied with changes in bladder function as outcome. Aim 3: Machine leaning to determine best parameters for Aim 1-2. Aim 4: Phase 1/2 clinical trial to evaluate bladder function.

- We have shown that neuromodulation of spinal cord in combination with conventional sensorimotor rehabilitation improves sensorimotor bladder function in SCI and TBI.
- We discovered that this multimodal approach has reduced improves bladder function.
- We propose a clinical trial to validate these discoveries.
- 6 subjects are enrolled. 5 completed stimulation sessions. We are moving closer to identify the best parameter to enhance micturition



Timeline and Cost

Activities CY	15	16	17	18
Aim1-2: Explore rehab parameters				
Aim 3: Define best parameters				
Aim 4: Apply best in Phase1/2 trial				
Estimated Budget ($K)	**$675**	**$504**	**$489**	**$489**

Updated: (Jul 15 2016)

Goals/Milestones

CY15 Goal – Pilot trial of MagStim + motor rehab for bladder function
☑ Obtained UCLA and DoD IRB approvals for Magnetic Stimulation
☑ Explore MagStim and bladder rehab parameter space
CY16 Goals – Machine Learning; Pilot trial (cont.)
☐Apply machine learning strategy to define best parameters in pilot
☑ Complete pilot trial with naïve subjects
CY17 Goal – Machine Learning; Pilot trial (cont.)
☐Develop best parameters for trial
CY18 Goal –Clinical trial of MagStim + motor rehab for bladder function
☐Complete clinical trial
☐Organize and analyze data for conferences and publications

Comments/Challenges/Issues/Concerns

- We have obtained UCLA IRB approval for the use of MagStim in bladder rehabilitation work proposed here.

4. **APPENDICES:**
    1. University of California, Los Angeles Institutional Review Board Approval.
    2. Publication.

Date: Wednesday, September 14, 2016 9:11:15 AM

Print Close

ID: IRB#14-000932 View: NEW 1.1 - Study Title and Key Personnel

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Study Title and Key Personnel

All items marked with a red asterisk (*) are required. Items without an asterisk may or may not be required depending on whether the items are applicable to this study.

1.0 ***Full Title of the Submission:**
Restoring Bladder Function by Spinal Cord Neuromodulation in SCI

1.1 **Protocol Version Date and/or Number:**

2.0 ***Working or Lay Title:**
Restoring Bladder Function by Spinal Cord Neuromodulation in SCI

3.0 **Principal Investigator:**

3.1 ***Name:** DANIEL LU
Degree(s): If degrees are not shown here, please add them to the next section, Section 1.1a/Item 1.0, which will then update the Principal Investigator's webIRB account information.
MD PhD

3.2 **UCLA Title:** Associate Professor

3.3 ***Will the Principal Investigator conduct the informed consent process with potential study participants?**

- (•) Yes
- ( ) No
- ( ) Not Applicable

3.4 ***Is the Principal Investigator an undergraduate student, graduate student, post-doctoral fellow, or resident physician?**

( ) Yes (•) **No**

3.4.1 **If you answered "yes" to the above question, indicate the Faculty Sponsor for this study.**

3.5 **UCLA Policy 900 defines types of UCLA employees who may be eligible to serve as a Principal Investigator. Check the policy to see if the Principal Investigator for this study needs an exception to the eligibility requirements.**

If an exception is needed, either attach the letter of exception here, or indicate a Faculty Sponsor in the above item.

Document Name	Document Version #
There are no items to display	

4.0 **Study Contact Person: Indicate the person, in addition to the Principal Investigator, who should receive all of the study correspondence.**
WILLIAM ALAYNICK

5.0 **List the key personnel and study staff below.**

Note: All personnel listed below are required to complete CITI training courses. HIPAA training is also required if personnel will be accessing protected health information.
Please make sure to have all key personnel update their webIRB profile, contact information.
Instructions on how to update the webIRB profile: Click here.

ID: IRB#14-000932

View: NEW 1.1a - Other Personnel

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Other Personnel

All items marked with a red asterisk (*) are required. Items without an asterisk may or may not be required depending on whether the items are applicable to this study.

1.0 Principal Investigator

1.1 **Name:** DANIEL LU
***Please type the Degree(s):** MD PhD

1.2 **Principal Investigator's UCLA Department:**
NEUROSURGERY

1.3 ***Protocol's UCLA Home Department:** NEUROSURGERY

This response defaults to the PI's payroll department. If you wish to affiliate this protocol with another department, please select the department from the list above.
For tips on effective search, please see guidance to the right.

2.0 If there will be other types of personnel working directly under the PI's supervision on aspects of the study, provide their name, title and institution, indicate their responsibilities, training and qualifications and complete Item 2.1.

Please also indicate, if applicable, whether that person will obtain consent, manage device accountability, have access to personally identifiable information and/or have access to the code key.

Note: If there will not be other types of personnel go to Item 3.0.

Name, title, institution Study role(s): e.g., conduct interviews/surveys, recruit participants, obtain consent, review records, etc.
View

For existing protocols: Item 2.0 has been modified and this item cannot be edited. When submitting an amendment please use the information found in the text box below to complete Item 2.0 above.
Briefly describe the other study personnel.

2.1 **Indicate the human subjects research training these personnel have or will receive. If training is required in a language other than English or if research is occurring in a location where research personnel do not have access to the internet (e.g., rural community without internet capability), please describe how human subjects training requirements will be fulfilled.**
Check all that apply:

- [x] CITI Training
- [x] UC HIPAA Training
- [ ] Other

2.2 **If you indicated "Other" to item 2.1, describe:**

3.0 ***Will any of the study procedures or analyses be contracted to a consultant or an organization?**

○ Yes ◉ **No**

3.1 **If yes, specify the consultant(s) and/or organization(s) and the work that they will do for the study.**

ID: IRB#14-000932 View: NEW 1.1b - Type of Study Review

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Type of Study Review

1.0 ***Indicate the level of risk involved with this study.**

(if there are multiple groups or phases associated with this study, select the highest level of risk.)

- ○ Minimal risk or no known risks - Click here for the OHRPP tip sheet on minimal risk.
- ◉ Greater than minimal risk

2.0 ***Indicate the type of review that you are requesting for this study.**

- ◉ IRB Review: Expedited or Full Board
- ○ Certification of Exemption from IRB Review

2.1 **If you indicated "IRB Review: Expedited or Full Board" as the type of review in item 2.0, select the IRB that you think best matches your research.**

	Name	Description
○	Medical Institutional Review Board 1	MIRB1 reviews general and internal medicine, infectious diseases and ophthalmologic research.
○	Medical Institutional Review Board 2	MIRB2 reviews oncology and hematology research.
◉	Medical Institutional Review Board 3	MIRB3 reviews neuroscience, neurology, psychiatric, drug abuse and dental research.
○	North General Institutional Review Board	NGIRB reviews research from the College of Letters & Science and the Professional Schools.
○	South General Institutional Review Board	SGIRB reviews social-behavioral research from the Schools of Public Health, Nursing, and Medicine.

***Please note*: The above requests are for initial routing purposes only. The final decision as to committee assignment and type of review, rests with OHRPP and/or the IRBs.**

ID: IRB#14-000932 View: NEW 1.2 - Conflict of Interest Information

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Conflict of Interest Information

1.0 * **Does the Principal Investigator, any of the key personnel, or their spouses, registered domestic partners, or dependent children, have a financial interest in the sponsor (profit, non-for-profit) of the research?**

○ Yes ◉ **No**

1.1 **If yes, attach a completed copy of the Financial Interests Form for each person who indicates a financial or related interest:**

Document Name	Document Version #
There are no items to display	

2.0 * **Does the Principal Investigator, any of the key personnel, or their spouses, registered domestic partners, or dependent children, have any financial interests related to the research sponsored by a government agency?**

○ Yes ◉ **No**

2.1 **If yes, attach a completed copy of the Financial Interests Form:**

Document Name	Document Version #
There are no items to display	

3.0 * **Indicate whether any of these financial interests have been submitted to or reviewed by the UCLA campus Conflict of Interest Review Committee (CIRC):**

◉ **Yes** ○ No

3.1 **If you have received a response from CIRC, attach it here:**

Document Name	Document Version #
Lu 2014-0038 follow up 3-13-2015.pdf	0.01
Lu 2014-0038 follow up 5-7-15 (1).pdf	0.01
Lu CIRC letter 3-19-14 DoD 14-000932.pdf	0.01

ID: IRB#14-000932 View: NEW 1.3 - Study Locations

Warning: Save your work at least every 15 minutes by clicking “Save” or “Continue.”

Study Locations

1.0 ***Indicate the locations where any research activities will be performed by the UCLA research team with participants and/or private information obtained.**

Check all that apply:

- [x] a. UCLA Sites or UCLA Health System Sites
- [ ] b. Off Campus (in California)
- [ ] c. Outside California (in the U.S.)
- [ ] d. Outside the United States ***See note at right**
- [ ] e. Internet

1.1 **If you selected b, c or d above, please provide your assurance that documentation of each site's permission to conduct the research at the site(s) will be obtained and maintained by the UCLA PI as applicable:**

Agree ☐

2.0 ***Is this a multi-institutional study (i.e., a collaborative project with other sites that have their own IRBs or principal investigators)?**
(Includes but not limited to UC MOU and CTSI MOU collaborations where UCLA IRB review is requested.)

○ Yes ◉ **No**

If no, please skip directly to the next page, do not complete the questions below.
If yes, please answer items 2.1-2.3:

2.1 **Will UCLA be responsible for the overall direction of the study at the other institutions?**

○ Yes ○ No

2.1.1 **Indicate the measures that will be taken to assure regulatory compliance at each site and that the following types of information will be communicated to the other sites: study procedures; modifications to the protocol and related documents; and safety updates, interim results and other information that may impact risks to study participants.**

Check all that apply:

- [ ] Conference calls or meetings with minutes distributed to each site
- [ ] Timely e-mail communications
- [ ] Postings on the study website
- [ ] Other

2.1.1.1 **If you chose "other", describe.**

2.1.2 **If you answered "yes" to item 2.1 above, please provide your assurance that the current IRB approval for each site(s) will be obtained and maintained by the UCLA PI as applicable:**

Agree ☐

2.2 **Will the UCLA principal investigator specified on this application be responsible for the data coordinating center?**

2.3 **Indicate the anticipated total number of study participants that will be enrolled across all of the institutions.**

ID: IRB#14-000932 View: NEW 1.4 - UCLA Sites or UCLA Health System Sites

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

UCLA Sites or UCLA Health System Sites

*Please complete this section if you indicated that your study is greater than minimal risk **AND** that research activities will be performed at UCLA Sites or UCLA Health System Sites.*

1.0 * **Indicate where study procedures or data collection procedures - that are greater than minimal risk - will be conducted.**

Check all that apply:

- [x] Clinical & Translational Research Center (CTRC)
- [x] Inpatient Medical Facility
- [x] Outpatient Treatment Facility/Private Office
- [ ] Public Area
- [ ] Research Laboratory
- [x] Other

1.1 **If you indicated "other", specify.**
Semel institute, 760 Westwood Plaza, Room 18-265

2.0 * **Indicate the resources available to handle potential emergencies related to study procedures that are greater than minimal risk.**

Check all that apply:

- [ ] This item is not applicable to this study
- [x] Basic Life Support (BLS) certified personnel
- [x] Advanced Cardiac Life Support (ACLS) certified personnel
- [x] Code Blue Team (hospital emergency response team)
- [x] Emergency crash cart
- [x] Paramedic Emergency Response Team (911)
- [ ] Suicide Protocol
- [ ] Other

2.1 **If you indicated "other", specify.**

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Project Identification Information

1.0 ***Type of Submission (Select one)**

- (•) Research Study
- ( ) Application for Approval of "Research Participant Pool" or recruitment database only

2.0

***Type of Submission (Select one)**

For Amendments, do not undo the response below. Undoing the response may remove sections of the original application.

- (•) New Submission
- ( ) Transfer of Ongoing Research from Another Site from Investigator moving to UCLA. Please complete Item 2.1.

2.1 **If you selected "Transfer of Ongoing Research" in Item 2.0 indicate the current status of the study and a brief summary of the work to date.**

3.0 ***Who developed this study?**

Check all that apply:

- [x] UCLA investigator
- [ ] Investigator from another institution
- [ ] Industry/Pharmaceutical Company
- [ ] Cooperative Group (e.g., Children's Oncology Group, AIDS Clinical Trial Group)
- [ ] Other

3.1 **If other, specify.**

4.0 **Review For and Reliance Upon External IRBs.**

***Indicate if one of the following applies to this study. (Select one)**

- (•) None of the options apply.
- ( ) UCLA IRB to serve as IRB of record for another institution.
- ( ) UCLA to RELY on another IRB.
  This includes reliance using UC MOU, CTSI, NCI, RAND, and Western IRBs.

5.0

***Is this study cancer related,** including the recruitment of individuals with cancer, collection of cancer human biological samples, specimens or data, or the recruitment of individuals because they are cancer survivors or at risk of developing cancer and/or involves gene therapy?

( ) Yes (•) **No**

Note: If you answered "Yes", you must submit an application to the Jonsson Comprehensive Cancer Center (JCCC) Internal Scientific Peer Review Committee (ISPRC). Click here for instructions for submitting to the ISPRC. The ISPRC approval notice or letter of exemption should be attached in Section 2.1/Item 6.2 of the webIRB application.

6.0

***Nurse Involvement:** Does this study involve any nursing time, effort, and/or resources at UCLA Health System sites, including as subjects, investigators, clinical care providers or data or specimen collectors?

( ) Yes ( ) No

ID: IRB#14-000932 View: NEW 2.2 - Lay Summary and Keywords

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Lay Summary and Keywords

Please provide the following information about your study.

1.0 ***Provide a brief lay summary describing this study. (limit 500 words).**

We are performing a study on individuals with spinal cord injury that has resulted in reduced bladder function. We will administer a mild magnetic stimulation to the skin over the spinal cord to activate the part of the spinal cord that controls the bladder. The participants will undergo stimulation and training to move their legs for several sessions as it appears this helps activate the bladder-related parts of the spinal cord. Then subjects will be examined for bladder function using specialized equipment used for measuring urine flow and bladder pressure. 24 subjects will be enrolled in this study of 4 years. Each subject will participate in the study for 6 months where they will have twice-a-week visits and be followed for 1 year afterwards.

2.0 ***List three to five keywords describing this study (separate the words with commas). The keywords may be used for identifying certain types of studies.**

Spinal cord injury, bladder, stimulation

3.0 ***Is this study conducted or supported by HHS (e.g., the National Institutes of Health, Centers for Control and Prevention, etc.)?**

○ Yes ◉ **No**

4.0 ***Is this study regulated by the Food and Drug Administration (FDA)?**

◉ **Yes** ○ No

4.1 **If yes, check all that apply:**

- ☐ Human Drugs
- ☑ Medical Devices
- ☐ Biological Products
- ☐ Food Additives
- ☐ Color Additives
- ☐ Other

4.1.1 **If Other, describe:**

ID: IRB#14-000932 View: NEW 2.3 - Methods/Procedures - Descriptors

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Methods/Procedures - Descriptors

Note: The items listed below are not an inclusive list of methods and procedures that may be used in research studies. The list only includes items that will trigger additional questions related to the research or are needed for the review process

1.0 * **Indicate all that apply to this study.**

☑	Audio, Visual or Digital Recordings
☐	Behavioral Observations (only applicable if you selected Exempt Category 2 in section 5.3)
☐	Certificate of Confidentiality
☐	Clinical Trial of a Drug, Biologic, Device or a Behavioral Intervention
☐	Community Based Research
☐	Controlled Substances (Schedule I or II)
☐	Deception or Partial Disclosure
☑	Devices/Diagnostics (including Humanitarian Devices - HUD)
☐	Drugs/Biologics/Dietary Supplements
☐	Expanded Access to Drug, Device or Biologic for Treatment Purposes (aka Compassionate Use, Treatment Use)
☐	Genetic Analyses/Genotyping
☐	Human Embryonic Stem Cells and/or Induced Pluripotent Stem Cells
☐	Human Gene Transfer/ Recombinant DNA
☐	Infectious Agents
☐	Non-FDA approved medical equipment used with UCLA hospital patients or research participants that operate under the UCLA Hospital License.
☑	Radiation (Standard of Care or Investigational use of radioactive materials or ionizing radiation)
☐	Substance Abuse Research (with Medication)
☐	Treatment in an Emergency Setting (with request to waive consent)
☐	**None of the above**

2.0 * **Will the study require services or resources owned/rented/operated or provided by the UCLA Health System (e.g. clinic and/or hospital visit(s), professional medical services, clinical treatment, diagnostics, labs, medical supplies, etc.)?**

Please direct any questions about this to the Clinical Trials Administration Office at coverageanalysis@mednet.ucla.edu.

◉ **Yes** ○ No

ID: IRB#14-000932 View: NEW 2.4 - Coverage Analysis

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Coverage Analysis

1.0 ***Will all protocol-required items and services that produce data for the study be funded by intramural or extramural funding/support?**

- (•) Yes - we will **not** bill participants or their insurers for any protocol-required items or services
- ( ) No - we will bill one or more protocol-required items or services to participants or their insurers
- ( ) Not Applicable – this is a non-interventional study (e.g., observational/registry/retrospective study without active treatment) that does not require additional visits, labs, items or services performed solely due to study participation

Note:

*If "**Yes**" is selected to the question above, then the corresponding "Research Only" cost language in the guidance to the right should be included in the ICF, and an abbreviated coverage analysis review is indicated.*

*If "**No**" is selected to the question above, then the "Mixed Cost" language in the guidance to the right should be included in the ICF, and a full coverage analysis review is indicated.*

*If "**Not Applicable**" is selected to the question above, then coverage analysis may not be applicable, and the corresponding "All Standard of Care" cost language in the guidance on the right should be included in the ICF.*

2.0 ***Is your study any of the following?**

- **Investigator-initiated study**
- **Expanded Access (aka Compassionate Use or Treatment Use)**
- **Humanitarian use device study**
- **Chemo/radiation therapy study**
- **UCLA IRB to rely on another IRB for this study**

( ) Yes (•) **No**

Note: *If you have selected yes, then continue with question 3.0 below.*

3.0 Please upload a copy of your study protocol below:

Document Name	Document Version #
There are no items to display	

The following item pertains to investigational drugs and devices only.

4.0 If the study participant or a third party payor (i.e., medical insurance/Medicare) will be billed for investigational products (i.e., investigational drugs and/or devices), attach any documentation to support these charges including any FDA letter(s) if available.

Document Name	Document Version #
There are no items to display	

ID: IRB#14-000932 View: NEW 6.1 - Funding and Other Study Characteristics

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Funding and Other Study Characteristics

1.0 ***Indicate the funding status for this study**.

- (●) Funded
- ( ) Application for funding is pending
- ( ) Departmental funding / Self funding / No funding

2.0 ***Check all that apply**:

- [x] The research will be conducted through the UCLA Clinical and Translational Research Center (CTRC)
- [x] The study will be supported by or conducted in collaboration with the U.S. Department of Defense (DOD)
- [ ] The study will be supported by or conducted in collaboration with the U.S. Department of Energy (DOE)
- [ ] The study will be supported by or conducted in collaboration with the U.S. Department of Justice (DOJ)
- [ ] The study will be supported by or conducted in collaboration with the U.S. Department of Education (ED)
- [ ] The study will be supported by or conducted in collaboration with the U.S. Department of Protection Agency (EPA)
- [ ] **None of the above**

2.1 **If you selected DOD, DOE, DOJ, ED, and/or EPA support/collaboration, please provide your assurances that you will review the additional requirements for research supported by the relevant federal agency.**

Agree [x]

Note: Please refer to the Federally-Supported Research section of the OHRPP guidance document: Funding Considerations for Federally-Funded and Industry-Sponsored Human Research.

ID: IRB#14-000932 View: NEW 6.2 - Funding - Description

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Funding - Description

Based on the response to section 6.1/item1, this study is or will be funded. Please provide the following information.

The Office of Contract and Grant Administration (OCGA) provides the list of funding sources used by webIRB in this section. Please check your OCGA paperwork to find the correct name of the funding source(s) for this study. Identifying the right funding source is important because:

▪ webIRB will auto-populate the designated funding source name on the approval letter for the study. Many funding sources require an accurate identification of their name on the IRB approval letter before they will release funding;
▪ The Office of Research Administration uses data from webIRB to generate funding reports.

Click here for tips on how to find the funding source name in webIRB.

1.0 **Identify the funding source(s).**
If a specific funding source has ended, do not delete it, instead please click Update next to the funding entry and **revise item 1.9**.

	Funding Source	Funding Source Information
View	DA-ARMY MEDICAL RESEARCH ACQUISITION ACTIVITY	

Name of the Funding Source	DA-ARMY MEDICAL RESEARCH ACQUISITION ACTIVITY
If other, specify	*No Value Entered*
UCLA PI named on the grant, contract, subcontract or gift:	DANIEL LU
Indicate the type of award:	Grant
Indicate the Grant Title:	Restoring Bladder Function by Spinal Cord Neuromodulation in SCI
Indicate the Award Number assigned by the funding source:	SCI130209
Indicate the description that applies to the source of funding named in the above item. If this is a subcontract, indicate the original source of funding:	Federal
If Other, specify	*No Value Entered*
Attach a copy of the funding proposal, subcontract, or scope of work.	**Document Name** DOD-Lu-10-2013.pdf **Document Version #** 0.01
Does the content of this IRB application differ from the activities described in the attached funding proposal, subcontract, or scope of work?	No
If yes, describe:	*No Value Entered*
Check this box to indicate that this specific funding has ended	*No Value Entered*

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Study Design

1.0 ***Check all that apply to the study design.**

- [x] **Direct subject contact ONLY** – The research activities involve direct contact with study participants (e.g., collection of data or specimens in person or via internet, phone, mail, etc.)
- [ ] **No direct subject contact** – None of the research activities involve direct contact with study participants and include only analyses of data, records and/or human biological specimens (e.g., medical record or other record review, study of specimens left over from clinical procedures).
- [ ] **BOTH Direct subject contact AND No direct subject contact** – Some of the research activities involve direct contact with study participants and some of the research activities involve analyses of data, records and/or human specimens obtained without contact with participants.

ID: IRB#14-000932 View: NEW 8.5 - Devices/Diagnostics and/or Humanitarian Devices

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Devices/Diagnostics and/or Humanitarian Devices

You indicated that this study includes devices/diagnostics and/or a Humanitarian Device (section 2.3/item 1.0). Please provide the following information.

1.0 For this study, list all Approved or Cleared (e.g., 510(k) or Premarket Notification (PMN); Premarket Application (PMA) devices that will be used within their approved labeling.
None

2.0 Complete only if one of the following apply:

- **The research involves *investigational use of an unapproved device*. The device is not approved by the FDA for marketing**
- **The research involves *investigational use of a marketed device*. The device will be used off label for an indication not in the approved labeling.**
- **The research involves use of a *device exempt from IDE regulations per 21 CFR 812(c).* Note: These exemptions apply in rare circumstances.**
- **The research involves a *humanitarian device.***

For additional information please refer to the OHRPP guidance documents on experimental drugs and devices.

Brand name of device	Investigational Devices Information

View MagStim

Trade (Brand) name of the device:	MagStim
Common (Generic) name of the device:	Magnetic Stimulator, Transcranial Magnetic Stimulator , TMS
Manufacturer of the device (if UCLA research lab, identify the lab):	MagVenture
Source of the device:	Manufacturer
If "Other" source, specify:	*No Value Entered*
FDA Reguatory Status of the Device	**Investigational Use of a Marketed Device:** The device will be used off-label for an indication and not in the approved labeling
Investigational Use of an Unapproved Device:	
Device is Exempt from FDA approval	
Humanitarian Use Device (HUD):	

3.0 Attach a copy of the Device Brochure for each device listed above, including a picture, if available. (if applicable)

Document Name	Document Version #
X100_productsheet.pdf	0.01

4.0 *Is the investigational Device(s) controlled by the PI?

(●) **Yes** ( ) No

4.1 If no, indicate by whom:

5.0

***By checking this box, I provide my assurance that all the person(s) who are authorized to manage the dispensation and accountability of the device have been identified in section 1.1/item 5.0.**

Agree ☑

6.0 *Describe the specific location where the device(s) will be stored and how the device(s) will be secured.
The device will be stored in a locked office.

ID: IRB#14-000932 View: NEW 8.8 - Audio, Visual or Digital Recordings

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Audio, Visual or Digital Recordings

You indicated that this study includes recordings (audio or visual) (section 2.3/item 1.0). Please provide the following information.

1.0 ***Who will transcribe the research tapes/recordings?**

Check as many as apply:

- [x] Members of the research team
- [ ] Persons outside the research team

2.0 ***Is the use of recordings an optional part of the research?**

- ( ) Yes
- (•) **No**

3.0

***Will individual study participants be able to review, edit, and erase the tapes/recordings of their research participation?**

- (•) **Yes**
- ( ) No

3.1 **If no, provide an ethical and scientific justification for NOT allowing study participants to review, edit, and erase the tapes of their research participation.**

4.0 **Transcription of Research Tapes/Recordings**

4.1 ***Type of media (Check as many as apply):**

- [ ] CD ROM
- [ ] DVD
- [x] Digital Files
- [ ] VHS tape
- [ ] Cassette or microcassette
- [ ] Handwritten files
- [ ] Other

4.2 ***Method of transmission (Check as many as apply):**

- [ ] Courier or mail with delivery confirmation
- [ ] Posted to a secure website
- [x] Email
- [ ] Other
- [ ] **Not Applicable**

4.3 ***Transcription Service (Check as many as apply):**

- [ ] Transcription service secures tapes in a secure locked area
- [ ] Transcription(s) sign confidentiality agreements
- [ ] Transmission of voice files and text files is encrypted and password protected
- [ ] Other
- [x] **Not Applicable**

4.3.1 **If you selected "other" for any/all of the above items, describe.**

ID: IRB#14-000932

View: NEW 8.11 - Radiation

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Radiation

Based on the response to section 2.3/item 1.0, you are seeking approval from MRSC committee. Please complete the following items.

1.0 * **Are the radiological procedures standard of care?**

Note: *Please review the guidance to the right before completing this question.*

○ Yes ◉ **No**

1.1 **If Yes, please provide the following information for EACH procedure:**
a. Type of standard of care radiological procedure.
b. Maximum number of times a subject will undergo this procedure in one year.
c. Building and room number where this procedure will be performed.
The MRSC review process cannot begin until all of the above-referenced information has been provided in the field below.

NOTE: If procedures include a radiopharmaceutical then an Investigational New Drug (IND) or Abbreviated New Drug Application (ANDA) must be described in Section 8.6.
Urodynamics under fluoroscopy of the upper pelvic region to visualize bladder. 7 procedures per year of less than 2 minutes of beam-on time per procedure.

Air kerma values for
1:fluoroscopy-guided procedures: 1.38 mGy
2. Peak skin dose: 2.18 mGy
3. Effective dose: 0.07 mGy
4. Maximum expected air kerma value for the urinary bladder imaging session: 4.0 mGy
Procedure will be performed at: 200 Medical Plaza, Suites 140, Peter Morton Medical Building, Los Angeles, CA 90095

7 total procedures at 1 per month for 6 months (0,1,2,3,4,5 and 6 months)

2.0 * **Will this study involve radiological procedures <u>beyond</u> the standard of care?**

Note: *If you have questions about what "beyond standard of care" means or questions about the forms to use in 2.1 below, or need help or additional information, please click* ***here****.*

◉ **Yes** ○ No

Important Note: If your study involves beyond standard of care radiological procedures that have not changed since previous approval through the MRSC/RDRC CARE system, upload the previously completed eight-page CARE Application in 2.2 instead of Forms A, B and/or C.

2.1 **If Yes and this is an *initial submission* or *an amendment involving changes to radiological procedures*, check all applicable administrations of radiation.**

- ☑ Radiation Producing Machines - *Form A required. Click* ***HERE*** *to download form.*
- ☐ Radiation Therapy - *Form B required. Click* ***HERE*** *to download form.*
- ☐ Radioactive Materials - *Form C required. Click* ***HERE*** *to download form.*

2.2 **Upload Forms A, B AND/OR C and other supporting documents.**

Document Name	Document Version #
Form A Rad Pro Mach - WebIRB.pdf	0.01

ID: IRB#14-000932 View: NEW 9.2 - Information about Study Data

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Information about Study Data

This information is needed to determine how you will best protect the confidentiality of data.

1.0 ***Indicate all that apply to the study data.**

Check all that apply:

- [ ] Obtained from a medical or clinical record
- [ ] Created or collected as part of health or mental health care
- [ ] Used to make healthcare or mental healthcare decisions and/or provided to other healthcare professionals
- [x] Research data will be entered into the participants' medical or clinical record
- [ ] **None of the above**

2.0 ***Is it reasonably foreseeable that the study will collect information that State or Federal law requires to be reported to other officials (e.g., child or elder abuse), ethically requires action (e.g., suicidal ideation), or is a reportable disease?**

○ Yes ◉ **No**

2.1 **If yes, explain below and include a discussion of the reporting requirements in the consent document:**

3.0 ***Indicate if any of the following are being obtained and used without any direct contact with study participants.**

- [ ] Records (Not medical)
- [ ] Human biological specimens
- [x] **None of the Above**

4.0 *Indicate all identifiers that may be **accessed or included in the research records** for the study**:**

- [x] Names
- [x] Dates
- [ ] Age (if over 89 years)
- [x] Postal Address
- [x] Phone Numbers
- [ ] Fax Numbers
- [ ] E-Mail Address
- [x] Social Security Number
- [x] Medical Record Number
- [ ] Health Plan Numbers
- [ ] Account Numbers
- [ ] License/Certificate Numbers
- [ ] Vehicle ID Numbers
- [ ] Device Identifiers/Serial Numbers
- [ ] Web URLS
- [ ] IP Address Numbers
- [ ] Biometric Identifiers (including finger and voice prints)
- [ ] Facial Photos/Images
- [ ] Any Other Unique Identifier (this does not include the code assigned by the investigator to identify the data)
- [ ] **None of the above**

4.1 **If social security numbers will be collected explain why they are necessary, how they will be used, how they will be**

ID: IRB#14-000932 View: NEW 9.2a - Privacy and Confidentiality

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Privacy and Confidentiality

Important Notes:

- **Privacy is about people.** Privacy refers to a person's wish to control the access of others to themselves.
- **Confidentiality is about data.** Confidentiality refers to the researcher's plan to handle, manage, and disseminate the participant's identifiable private information.

See OHRPP Quick Guide: Protecting Privacy and Maintaining Confidentiality

1.0

***Privacy: How will the investigator maintain privacy in the research setting(s)? (e.g., interviewing participant in a room or area where conversations cannot be overheard by others, or conducting medical procedures in an examination room, or behind a curtain in an emergency room).**

Patient will be consented in the clinic in a private room, behind closed doors. Subsequent testing will be conducted in a clinical research laboratory space in UCLA CTRC.

2.0

***Confidentiality: If the protocol will collect and maintain identifiable data, explain how the planned safeguards to maintain confidentiality of identifiable data and data security are appropriate to the degree of risk from disclosure.**

Note: Other sections of the application (e.g., Sections 9.3, 9.3a, 9.4, 9.5, and 15.3) will request specifications such as identification of persons who will have access to code keys or measures to comply with HIPAA requirements.

All data collected will be placed on password protected files on an encoded hard drive. The data will be de-identified and coded key placed in a password protected file and on a separate encoded hard drive. The hard drives will be placed in a locked cabinet in the office of the PI in CHS, the door to the office is locked with only access from the PI, CHS is located behind security access points during off hours.

ID: IRB#14-000932 View: NEW 9.3 - Data Security

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Data Security

You indicated that the study team will have access to personally identifiable or coded information (Section 9.2/item 5). Please complete the following items.

1.0 ***Do you agree to follow the OHRPP Data Security in Research guidance and procedures?**

- (•) Yes
- ( ) I have an alternate equally effective plan (*Note: The plan must be attached to item #2.1*)

2.0 ***Do you have a data security plan for this study?** *(Note: a plan is not required for all studies; it may be recommended in some instance).*

( ) Yes (•) **No**

2.1 **If yes, attach it here:**

Document Name	Document Version #
There are no items to display	

3.0 ***Indicate all that apply to personally identifiable information or codes during conduct of the study:**

- [x] The data and/or specimens will be coded
- [ ] The personal identifying information will be removed and destroyed
- [ ] Personally identifying information will be maintained with the data and/or specimens

3.1 **If you indicated that the personal identifying information will be removed or destroyed or that the data/specimens will be coded, provide the following information:**

- **The process for removing and destroying the personal identifying information or for coding the information, and**
- **Indicate who will perform the task**

Personally identifying information will be coded. Coding will be performed by a random number generator and code assigned to study participants. The coded key will be kept on a printout in a locked file cabinet behind a locked office door in CHS.

4.0 ***Will coded or personally identifiable data be collected, transmitted or stored via the internet?**

( ) Yes (•) **No**

4.1 **If yes, indicate all that apply:**

- [ ] A mechanism such as Survey Monkey, Zoomerang, or an e-mail anonymizing service will be used to strip off the IP addresses for data submitted via e-mail.
- [ ] The data will be encrypted.
- [ ] A firewall will be used to protect the research computer from unauthorized access.
- [ ] Controlled access privileges will be used on the hardware storing the data.
- [ ] Other.

4.1.1 **If you indicated "Other", describe:**

5.0 ***Provide your assurances that if there is a data security breach for this study, the PI will notify the IRB and your department's IT Compliance Coordinator.**

Agree [x]

ID: IRB#14-000932 View: NEW 9.4 - Data Security Plan - During the Study

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Data Security Plan - During the Study

You indicated that data and/or specimens for this study will be coded (Section 9.3/item 3). Please complete the following information.

1.0

During the study indicate **how data will be stored and secured** including paper records, electronic files, audio/video tapes, specimens. Specify how the **code key** will be securely maintained, as applicable.

Check all that apply:

1.1 ***Electronic Data**

- [x] Encryption or password protection software will be used
- [ ] Secure network server will be used to store data
- [ ] Stand alone desktop computer will be used to store data (not connected to server/internet)
- [ ] A contracted outside vendor will store the code key. The vendor will have a business associate agreement with UCLA.
- [ ] Other
- [ ] **Not Applicable**

1.2 ***Hardcopy Data, Recordings and Specimens**

- [x] Locked file cabinet or locked room with limited access by authorized personnel
- [ ] Locked lab/refrigerator/freezer with limited access by authorized personnel
- [ ] The code key will be kept in a locked file in a locked room
- [ ] The coded data and/or specimens will be maintained in a different room
- [ ] Other
- [ ] **Not Applicable**

1.3 **If you indicated "Other" in item 1.1 or 1.2 above, describe here.**

2.0

***By checking this box, I provide my assurance that all the person(s) who will have access to the code key have been identified in section 1.1 or section 1.1a.**

Agree [x]

ID: IRB#14-000932 View: NEW 9.5 - Data Security Plan

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Data Security Plan

You indicated that the study will have access to personally identifiable or coded information (Section 9.2/item 5). Please complete the following items:

1.0 ***After the study is completed**, indicate how the data codes and/or personal identifying information will be handled.

Check all that apply:

- [x] All data files will be stripped of personal identifiers and/or the key to the code destroyed.
- [ ] All specimens will be stripped of personal identifiers and/or the key to the code destroyed.
- [ ] Personal identifiers and/or codes linking the data and/or specimens to personal identifiers will be maintained for future research.
- [ ] Audio or Video recordings will be transcribed and then destroyed or modified to eliminate the possibility that study participants could be identified.
- [ ] Photos or Images will be modified to eliminate the possibility that study participants could be identified.
- [ ] Restricted use data will be destroyed or returned to the source.

1.1 **If you indicated that personal identifiers will be maintained for future research, provide the following information:**
a) How the information will be securely handled and stored
b) assure confidentiality, and
c) who will have access to the identifiers and/or codes.

2.0 **Describe any additional steps, if any, to be taken to assure that the subjects' identities and any personal identifying information are kept confidential.**

ID: IRB#14-000932 View: NEW 9.8 - Data and/or Specimens for Possible Future Use

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Data and/or Specimens for Possible Future Use

You indicated that prospectively collected data and/or specimens would be stored for future use (Section 9.2/item 5.1). Please provide the following information.

1.0 ***Specify what information directly or indirectly linked to the subject will be provided with data and/or specimens to other investigators.**

Check all that apply:

- [x] No subject identifiers (The data/specimens are anonymous; no one including the investigator could identify the person from whom the materials were gathered.)
- [ ] The data will be coded (A code links the data/specimens to the study participants. A key to the code exists.)
- [ ] Personal Identifying Information
- [ ] **Not applicable, the data will not be shared outside the study team.**

2.0 **Distribution Rules: Describe the criteria used to determine the adequacy of requests to obtain data and/or specimens (e.g., the type of researchers that will be eligible to receive data):**

ID: IRB#14-000932 View: NEW 10.1 - Study Summary - Research Study

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Study Summary - Research Study

1.0

Study Materials: As applicable to this study, attach the following:

- **Protocol, Dissertation Proposal or Study Plan**
- **Preliminary Data**
- **Surveys, Questionnaires or other instruments to be used with study participants**
- **References**

Document Name	Document Version #
Procedures_Clean.docx	0.01
Procedures_Redlined.docx	0.01
References Cited DoD Bladder.docx	0.01

2.0 ***Specific Aims: Indicate the purpose of the research, specifying the problems and/or hypotheses to be addressed.**

Specific Aim 1: Determine the optimal stimulation parameters to enable micturition in SCI subjects.
We hypothesize that optimal spinal cord stimulation parameters exist that are selective or specific for micturition. We will test combinations of spinal cord levels (T10-L4, coccyx levels) and stimulation frequency (1-30 Hz) administered twice weekly for 24 weeks. A machine learning algorithm will guide subsequent stimulation parameters (Aim 3). Subjects will be evaluated at each session for urine flow, volume, detrusor pressure, and self-assessments of quality of life and urinary function. Formal urodynamics will be tested monthly and at the conclusion of the 24-week study.
Specific Aim 2: Determine the minimum training conditions to enable micturition in SCI subjects.
We hypothesize that the naïve post-injury spinal cord requires some minimal stimulation quality and duration to re-awaken dormant micturition neural circuitries. Using the optimum stimulation parameters determined in Aim 1, we will test a pre-training regimen in naïve SCI subjects. In addition to determining the minimum number of sessions, we will examine the types of locomotor training that will best enable micturition function, administered twice weekly for 24 weeks. Assessments will be as in Aim 1.
Specific Aim 3: Application of machine learning strategies for determining the optimal stimulation and training parameters to induce micturition in SCI subjects.
We hypothesize that existing machine learning techniques for locomotion can be adapted to determine the optimal stimulation parameters for micturition. Data from each session will be added to a machine learning algorithm database to determine the most effective parameters for the most recent session and guide stimulation parameters for subsequent sessions. At the conclusion of this Aim, the most effective conditions for urination will be determined and used in the clinical trial.
Specific Aim 4: Application of the optimal stimulation and training parameters for inducing micturition in SCI subjects in a pilot clinical trial.
This Aim will combine the optimal stimulation and training parameters from Aims 1-3. In this Aim we will test the hypothesis that an optimized stimulation paradigm can improve micturition function in naïve, SCI subjects. 12 subjects will be tested twice weekly for 24 weeks. Urodynamics and self-assessments will be as Aim 1.

3.0 ***Background and Significance: Provide a summary of the background for this study and explain how it will contribute to existing knowledge.**

For greater than minimal risk biomedical studies, include preliminary data. If necessary, attach in Item 1.0 graphs or tables used to convey information. If there no preliminary data are available, briefly indicate why this proposed study is a reasonable starting point.

Little progress has been made in developing any intervention that will enhance bladder function after a SCI. However our team has performed a systematic progression of experiments in animals and patients showing that several forms of electrical spinal cord stimulation can detect and improve spared function (Table 1, Figure 1). We have implanted an epidural stimulation (EDS) electrode array over the lumbosacral spinal cord in 4 human subjects with a motor complete SCI. Each has gained some voluntary control of urinary voiding in the absence of EDS (1, 2). Furthermore, we have preliminary data showing that single-electrode TESS or EDS of the cervical spinal cord can improve fine motor function of the upper limb in human subjects with incomplete quadriplegia. The critical question here is whether TESS can be used to enable spared function of sacral neuromotor networks, i.e., neural networks related to bladder function in animals and humans (3-6). The potential impact of these therapeutic interventions on the lives of individuals with urinary incontinence cannot be overestimated (7, 8). Development of magnetic stimulation to activate spared, but silent, spinal cord pathways related to bladder function in humans could represent the beginning of a paradigm shift in the rehabilitative approach to bladder incontinence as a result of SCI and potentially other neurologic injuries or stroke (9-12). It could also provide new pathways toward more advanced technologies to further enable more potential success in improving bladder function.
As we have observed with studies of the lumbosacral spinal cord in completely paralyzed SCI subjects, future development of technical capabilities to use neuromodulation of the lumbosacral spinal cord for evaluation and enabling of spared function will undoubtedly enhance our ability to improve therapies for bladder function after paralysis. By discovering spared function and revealing the potential for treatments, the cost-savings from the proposed translational studies could include reduced assistive daily care costs, increased employment, and improved quality of life—especially for incomplete SCI patients who account for the majority

ID: IRB#14-000932 View: NEW 11.1 - Characteristics of the Study Population

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Characteristics of the Study Population

1.0 ***Is this an observational or ethnographic study for which the number of participants observed or interviewed cannot be determined in advance.**

Yes **No**

2.0 **If you answered "no" to item 1.0, indicate the maximum number of study participants you hope to enroll:**

24

3.0 **How many participants do you expect you will need to recruit, consent and/or screen to meet the target number above?**

48

4.0 ***Indicate the specific inclusion criteria for enrollment of each of the groups of research participants in this study. If there are any inclusion criteria based on *gender, pregnancy/childbearing potential, race, ethnicity or language spoken*, explain the nature of and scientific rationale for the inclusions.**

1. Male 18-75 years; This is required to have one urethral anatomy. Secondarily, more males have spinal cord injury, especially in veteran populations.
2. At least 1 year post-injury;
3. Non-progressive SCI at C2-T8 (non-conus injury);
4. Motor Complete ASIA (A, B, C or D);
5. Neurogenic bladder requiring clean intermittent straight catheterization;
6. Able to attend twice weekly testing sessions for 6 months.
7. Have intact lower extremity anatomy and able to use lower extremity for assistive standing and stepping. This is required to assess the quality of motor-function-activating spinal cord stimulation.

5.0 ***Indicate the specific exclusion criteria for each of the groups of research participants in this study. If there are any exclusion criteria based on *gender, pregnancy/childbearing potential, race, ethnicity or language spoken*, explain the nature of and scientific rationale for the exclusions.**

1. History of autonomic dysreflexia;
2. Ventilator dependency;
3. Musculoskeletal dysfunction, unhealed fracture, pressure ulcer, active infection;
4. Clinically significant depression or ongoing drug abuse;
5. Received botox injection, or bladder surgery (suprapubic access, Brindley procedure, etc.); 6. Prostatic hypertrophy or bladder outlet disorder;
7. Cardiopulmonary disease that precludes lower extremity training or rehabilitation.

6.0 ***How (chart review, additional tests/exams for study purposes, etc.), when and by whom will eligibility be determined?**

We will recruit subjects who have sustained a cervical SCI at least one year prior to enrollment to participate in the proposed experiments; specifically, individual subjects with non-progressive SCI at C2-T8 (non-conus injury), classified as motor complete (A or B) or incomplete (C or D) on the ASIA SCI scale; specifically SCI subjects with neurogenic bladder who are performing urethral catheterization procedures for bladder care will be recruited. With these criteria, we are screening for subjects with hypertonic or hyperreflexive neurogenic bladder and are excluding subjects with areflexive or hypotonic bladder (conus lesions). The reason for this is that our strategy depends on an intact spinal cord-bladder circuitry.

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Characteristics of Study Population

1.0 * **Indicate the age range of the study participants.**

Check all that apply:

- [ ] 0 to 6 years
- [ ] 7 to 11 years
- [ ] 12 to 17 years
- [ ] 17 or younger **in California** who can consent for themselves - see note below
- [ ] 17 or younger **outside California** who can consent for themselves - see note below
- [x] 18 years or older

NOTE:

- For additional information on minors **in California** who are permitted to consent for themselves please refer to the section "Legal Exceptions Permitting Certain Minors to Consent" in the OHRPP Guidance document, Child Assent and Permission by Parents or Guardians
- For additional information on minors **outside of California** who are permitted to consent for themselves please refer to the section "Exceptions Outside of California" in the OHRPP Guidance document, Child Assent and Permission by Parents or Guardians

2.0 ***Indicate if any of the following populations/specimens will be specifically recruited/obtained for the study.**

- [x] Adults who are competent to give informed consent
- [ ] Adults unable to give informed consent
- [ ] Adults with diminished capacity to consent
- [ ] Fetal Tissue
- [ ] Neonates
- [ ] Participants Unable to Read, Speak, or understand English
- [ ] Pregnant Women/Fetuses
- [ ] Prisoners
- [ ] UCLA Faculty/Staff
- [ ] UCLA Students
- [ ] Wards
- [ ] **Unknown/Not Applicable**

3.0 * **Is it possible that there may be non-English speakers enrolled in this study or children whose parents are non-English speaking?**

(•) **Yes** ( ) No

ID: IRB#14-000932 View: NEW 14.1 - Risks & Benefits

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Risks & Benefits

Benefits

1.0 ***Are there any potential direct benefits (physical, psychological, social or other) to study participants?**

○ Yes ◉ **No**

1.1 **If yes, describe.**
Potential Benefits of the Proposed Research to the Subjects and Others: There may be no benefit. Exercise and rehabilitation has been considered beneficial for people with SCI who are confined to a wheelchair, as immobilization can contribute to secondary pathologies such as muscle contractures, decreased cardiovascular health, pressure sores, and muscle atrophy. Because individuals respond differently, it cannot be predetermined if this research will be beneficial to a specific type of subject. Potential benefits may include: increase in cardiovascular fitness, decrease in spasticity, and/or an improved ability to utilize lower extremity function.

2.0 ***Describe the potential benefits to society including the importance of the knowledge to be gained.**
Importance of the Knowledge to Be Gained: The proposed experiments will demonstrate whether a new strategy of neuromodulation via non-invasive spinal cord stimulation can be used to improve bladder function. Positive demonstration of the proof-of-principle of the neuromodulatory strategy would almost certainly result in significant improvements in the quality of life after a spinal cord injury and could significantly reduce the cost of healthcare for these individuals by making them more independent. The knowledge gained also will demonstrate whether a medical stimulation device that is presently approved for other neuromotor dysfunctions can be used to improve bladder function. In addition we will learn whether a newly developed technology, transcutaneous stimulation, can be used to neuromodulate the spinal cord to improve bladder function. To date, there has been virtually no progress in improving bladder function after spinal cord injuries. The potential of the proposed studies are extremely positive. Given the magnitude of the scientific evidence from which the neuromodulatory strategy has evolved combined with our preliminary evidence in humans and rats, the potential gain that can be realized by so many impaired individuals given the modest total cost to be incurred in this grant cannot be denied.

Risks

3.0 ***Indicate the potential risks/discomforts, if any, associated with each intervention or research procedure.**

Additionally discuss any measures that will be taken to minimize risks. If data are available, estimate (a) the probability that a given harm may occur, (b) its severity, and (c) its potential reversibility. The information provided should be reflected in risks section of the informed consent documents.

If this is an exempt study and there are no risks, indicate N/A. Otherwise, please see the help text.
Risk from Transcutaneous Stimulation: The transcutaneous stimulation device is noninvasive and procedure has been approved by the UCLA Institutional Review Board (IRB#11-001720). There is a minor risk of discomfort during the stimulation procedure that stops after stimulation. There is also a minor risk of skin irritation with the adhesive electrode during stimulation. If this occurs, another site can be used, or testing postponed until skin heals.
Risk from Interventions and Experimental Procedures: Because subjects must meet the criteria listed above, we expect all subjects to be in good health. The studies described may involve the following physical risks and/or discomforts: 1) increased respiration or shortness of breath; 2) increased heart rate; 3) muscle and joint soreness; 4) lowering or elevation of blood pressure; 5) dizziness; 6) skin irritation from recording electrodes, or hand placements of trainers; 7) skin abrasion from hand placements of trainers; and 8) muscle strain or joint sprain from movement, or from the force exerted by the trainers.
Most subjects will have increased respiration and heart rate due to an increase in activity. However, we do not expect the increase in respiration and heart rate to be greater than what is normally experienced during regular exercise. Many SCI subjects will likely sustain skin irritation from the recording electrodes, or hand placements of the trainers. These conditions are considered to be minimal risks and are reversible. There is some chance that subjects may sustain muscle and joint soreness, lowering or elevation of blood pressure, dizziness, or skin abrasion from hand placements of the trainers. If these events occur the experiment would cease immediately. These conditions are considered to be minimal risks and are reversible.
It is highly unlikely that a subject would feel chest pain or high blood pressure would occur that did not resolve within several minutes. These events have not occurred in our past experience. Blood pressure will be monitored throughout the testing session at 1-5 minute intervals by arm blood pressure cuff. However, if this did occur the individual would be immediately transported to the University of California, Los Angeles Emergency Unit and Drs. Lu, and/or Denis, and/or Niu notified. It is also highly unlikely that a subject would suffer a muscle strain, joint sprain, or fracture from upper extremity physical therapy. These conditions are

ID: IRB#14-000932 View: NEW 15.1 - Data & Safety Monitoring Plan

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Data & Safety Monitoring Plan

1.0 * **Is a Data and Safety Monitoring Plan (DSMP) required by the funding agency or other entity?**

○ Yes ◉ **No**

ID: IRB#14-000932 View: NEW 15.2 - Data & Safety Monitoring Plan (continued)

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Data & Safety Monitoring Plan (continued)

Important Note:

All interventional studies involving more than minimal risk must include a Data and Safety Monitoring Plan (DSMP). A DSMP is a plan established to assure that each research study has a mechanism for appropriate oversight and monitoring of the conduct of the study to ensure the safety of participants and the validity and integrity of the data. The DSMP should indicate specifically whether or not there will be a formal Data Safety Monitoring Board (DSMB) or Data Monitoring Committee (DMC).

Most, but not all studies (i.e., non-interventional studies) undergoing full board review will require a DSMP. You will need a DSMP if any of the following apply:

1. *This is a Phase I, II or III clinical trial*
2. *This is an investigator initiated trial (Section 2.1/item 3.0)*
3. *This study involves treatment in an emergency setting (Section 2.3/item 1.0)*
4. *A Data/Safety Monitoring Plan is required by the funding agency (Section 15.1/item 1.0)*
5. *This study is greater than minimal risk (Section 1.1b/item 1.0)*

1.0 ***Indicate who will be responsible for overseeing the study safety. Check all that apply.**

- ☐ The Principal Investigator
- ☑ Designee of the Principal Investigator
- ☐ The DSMP includes at least one person who is not associated with the study
- ☐ A formally constituted Data and Safety Monitoring Board (DSMB)
- ☐ Medical monitor designated by the sponsor
- ☐ Other

1.1 **If you indicated that a designee would be responsible for overseeing the study safety, or that the DSMP would include at least one person not associated with the study, provide the name(s) of this individual (s). Also, provide a brief explanation of why this person(s) would be appropriate in this role(s).**

Dr. Daniel Denis and Niu will be responsible for overseeing the study safety, along with the External Research Monitor, Victor Chang, MD.

The Research Monitor, Victor Chang, MD (Director, Spine Research, Department of Neurosurgery, Henry Ford West Bloomfield Hospital, West Bloomfield, Michigan) is responsible to oversee the safety of the research and report observations/findings to the IRB of Record or a designated official. The Research Monitor will review all unanticipated problems involving risk to volunteers or others associated with the protocol and provide an unbiased written report of the event to the IRB of Record. The Research Monitor may discuss the research protocol with the investigators, interview human subjects, and consult with others outside of the study about the research. The Research Monitor shall have authority to stop the research protocol in progress, remove individual human subjects from the study, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report. The Research Monitor is responsible for promptly reporting their observations and findings to the IRB.

1.2 **If you indicated "other," describe or indicate where the information can be found in the attached protocol.**

2.0 ***Provide your assurance that information about serious, unanticipated problems related to the study (e.g., adverse events, incidents and violations) will be reported to the IRB within the time frames specified by the Summary Sheet of Reporting Requirements.**

Agree ☑

ID: IRB#14-000932 View: NEW 16.1 - Payment, Costs, and Injury

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Payment, Costs, and Injury

1.0 * **Indicate what the participants will receive for their participation in the study.**

Check all that apply.

- [ ] No payment will be provided
- [x] University check
- [ ] Course Credit
- [ ] Cash
- [ ] Gift Cards/Bruincard Deposit
- [ ] Non-Monetary Gifts or Services
- [ ] Other (including vouchers for parking)

1.1 **If you selected Non-Monetary Gifts or Services or Other, describe:**

1.2 **If you selected *Cash* and/or *Gift Cards/Bruincard Deposit* please specify the estimated total amount of money you will require to pay all participants during the length of the entire study. This information is required by UCLA Business and Finance Services (BFS), the office that will provide the cash/gift cards for payment.**

2.0 **If study participants will receive financial or other payment for their participation in the study, please provide the following information:**

- If applicable, the amount each participant will receive and the payment schedule to be followed including whether partial payment will be provided when the participant does not complete the study.
- If there are different plans for different populations or sub-studies, specify the groups and describe the plans.
- If families or children will be involved in the research, clarify how the payments, items or services will be apportioned.

Subjects will receive $35 per visit, two visits per week, for either 24 or 27 weeks.

Partial payment will be provided when the participant does not complete the study.

3.0 * **Will subjects** incur **any financial obligations from participation in the study?**

( ) Yes (•) **No**

3.1 **If yes, describe:**

4.0 * **Indicate below that you are familiar with UCLA policy related to treatment and compensation for injury and that you will use in the consent form for this study the appropriate UC required statement describing "Treatment and Compensation for Injury."** Click here **to access the UCLA policy: Treatment and Compensation for Research Related Injury.**

Note: *Select* ***Not Applicable*** *if study is minimal risk.*

- (•) Agree
- ( ) Not Applicable

ID: IRB#14-000932 View: NEW 17.1 - HIPAA Authorization

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

HIPAA Authorization

According to your responses to section 9.2/item 1.0, this study uses protected health information. Please provide the following information.

1.0 ***Indicate all that apply to use of or disclosure of PHI in this study:**

- [x] All UC participants will sign a UC HIPAA Research Authorization for Release of Personal Health Information for Research.
- [x] **Another Institutions' Healthcare Authorization** for Release of Health Information will be used **or a waiver** for release of health information will be granted **from another Institution**.
- [x] **A Waiver of HIPAA Research Authorization** is requested for **screening** using UC medical records. I assure that the PHI collected for this study will not be reused or disclosed, except as indicated in this application.
- [ ] **A Total Waiver of HIPAA Research Authorization** is requested for the entire study. I assure that the PHI collected for this study from UC records will not be reused or disclosed, except as indicated in this application.
- [ ] **Limited Data Set with a Data Use Agreement** will be obtained from UC medical records. I assure that I will follow the data security plan outlined in this application to protect the identifiers from improper use or disclosure.
- [ ] **None of the above. This study will be conducted outside the United States**

2.0 ***Indicate to whom or where you will grant access to personal identifying information (including PHI) as part of the study process:**

- [x] There is no plan to share identifiers outside the study team
- [ ] The study sponsor; on site only (if there is more than one study sponsor, specify below).
- [ ] A foreign country or countries
- [ ] Other

2.1 **If you checked "other", "a foreign country or countries", or if "there is more than one sponsor", specify.**

3.0 ***The investigator's agreement is needed to the following:**

- The protected health information requested is the minimum necessary to meet the research objectives

- The protected health information that is obtained as part of this study will not be used or disclosed to any other person other than study personnel or to the parties listed in item Section 17.1/item 2, except as required by law.

- Study Sponsors will **not** be provided with personal identifying information (including PHI) to take from the study site at any time, including the end of the study.

- Data and specimens shared with outside entities, such as study sponsors, will be coded or de-identified.

Agree [x]

ID: IRB#14-000932 View: NEW 17.2 - HIPAA - Waiver of Authorization

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

HIPAA - Waiver of Authorization

According to your responses to Section 17.1/item 1, a waiver of authorization is requested. Please provide the following information.

In addition to the information that will be requested later in this application for a waiver of informed consent, HIPAA requires the following information for a waiver of authorization:

1.0 * **Indicate why the research could not be practicably conducted without access to and use of the protected health information.**

Check all that apply.

- [x] The PHI is needed to identify potential participants with a specific medical condition
- [x] It would not be feasible to individually contact the large numbers of potential subjects in the study
- [ ] It would not be possible to locate many of the individuals whose records would be used for the study
- [ ] Many of the individuals, whose records would be used for the study, are now deceased
- [ ] Other

1.1 **If you checked "other", specify.**

ID: IRB#14-000932 View: NEW 18.1 - Identification/Recruitment Methods

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Identification/Recruitment Methods

1.0 * **How will you identify and/or recruit participants for this study.**

Check all that apply:

- [ ] Advertisements/Flyers/Information Sheet/Internet Postings
- [x] Direct recruitment of potential study participants (e.g., physicians talking with their own or clinic patients about the study, contact between the study team and potential subjects in person, on the phone or on the internet, etc.)
- [ ] Random or Other Probability Sampling
- [ ] Recruitment Letters/Emails
- [x] Referrals (e.g., referrals from non-investigator healthcare providers, snowball sampling, participants referring other participants, etc.)
- [x] Review of medical records to identify potential research participants
- [ ] Review of publicly available records
- [ ] Review of other records
- [ ] Participant pool for which potential research participants have given permission for future contact
- [ ] Potential Study Participants are identified from another IRB approved study or IRB approved screening protocol
- [ ] Other

ID: IRB#14-000932 View: NEW 18.2 - Recruitment Methods

Warning: Save your work at least every 15 minutes by clicking “Save” or “Continue.”

Recruitment Methods

1.0 Please upload copies of your recruitment materials below. This includes advertisements, flyers, internet postings, recruitment scripts and letters/emails.

Document Name	Document Version #
There are no items to display	

Ads/Flyers/Info Sheets/Internet Postings

2.0 If you have indicated that study participants will be recruited with advertisements/flyers (Section 18.1/Item 1.0), please indicate the type of media that will be used (e.g., newspaper, radio, internet, etc.) and/or where information will be posted or distributed.

Direct Recruitment

3.0 If you have indicated that participants will be recruited through direct contact (Section 18.1/Item 1.0), please provide the following information:

- A description of how, when, and where initial contact would be made (e.g. in a public setting, in a waiting room, via a phone call, via a letter, via the internet, etc.)
- If applicable to the study, indicate how the potential research participant's privacy will be maintained.
- Who will make the contact (e.g. the investigator, a patient's physician, etc.)

Recruitment of individuals with SCI will be from VA Greater Los Angeles, VA Long Beach, and UCLA Health Care System. Additional referrals will be generated from treating physiatrists, urologist, neurologists, and other clinicians at referral sites.

Initial contact will be made in person by a potential participant's physiatrist, urologist, neurologists or other clinician during an office visit. The potential participant will be provided with contact information, e.g. phone number, email address, of the PI, Dr. Lu. Once Dr. Lu has been contacted, he and his staff will schedule a visit by the potential subject for potential consent and enrollment.

3.1 If you will be directly recruiting potential participants who are your patients, students, laboratory workers or any others with whom you have a relationship of authority or unequal power, describe what measures you will put in place to avoid those approached from feeling pressured or unduly influenced to participate in the study.

Recruitment Letters/Emails

4.0 If you have indicated that recruitment letters will be distributed to participants (Section 18.1/item 1.0), please indicate who will send out the recruitment letter (i.e. will it be the investigator or other persons who have authorized access to the information), how inquiries will be handled, and if there will be follow-up contacts.

Referrals

5.0 If you have indicated that study participants will be identified from referrals (Section 18.1/item 1.0), please indicate the source of the referral (e.g., friends, other participants, healthcare providers) and how the referral will be elicited.
The SCI patients with neurogenic bladder with the above inclusion/exclusion criteria will be identified by their treating physician at any referral site, or by the SCI database at UCLA. Recruitment of subjects will be generated from database of SCI subjects in UCLA Health Care System. Additional referrals will be generated from treating physiatrists, urologist, neurologists, and other clinicians at referral sites. Subjects who may qualify will be informed about the study by their treating physicians and given contact information for the PI, to call if they are interested. If they meet chart-review based inclusion and exclusion criteria they will be invited to attend an appointment for informed consent before further procedures are conducted.
Once the patients have been identified, they will be given the opportunity to meet with the principal investigator in order to discuss the purpose and the procedures involved in the trial. Dr. Lu will complete a chart review along with medical history and neurological examination to determine the medical eligibility for each SCI subject. Additionally, Dr. Lu will determine study eligibility based on the inclusion and exclusion criteria. Experimental testing and training interventions will be initiated after the subject has been evaluated and determined to be in compliance with the selection criteria. The subjects will not be concurrently enrolled in any other experimental studies. All subjects will sign an informed consent that has been approved by the UCLA Institutional Review Board

ID: IRB#14-000932

View: NEW 18.7 - Review of Medical Records

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Review of Medical Records

1.0 ***You have indicated that potential research participants will be identified from medical records (Section 18.1/item 1). Indicate the specific records to be reviewed and the information that will be obtained to identify potential participants for this study.**

Clinic records of UCLA spine surgeons will be assessed for patients with SCI. After identification of the subject with SCI, the records will be assessed for satisfaction of enrollment criteria. If the enrollment criteria are satisfied, the patient may be contacted for enrollment.

1.1 **If you have a data sheet summarizing the information that will be obtained from the records, you can upload it here instead of listing the information above.**

Document Name	Document Version #
There are no items to display	

Federal and State Regulations require that the IRB review the information below to determine if a waiver of consent and authorization is appropriate for use of medical record information for recruitment purposes.

2.0 ***Do you assure the following?**

- The information that will be reviewed is the minimal necessary to identify potential research participants for this research.
- The information that will be obtained for identification of participants will not be reused or disclosed outside the research team, except as required by law.
- All study personnel will comply with HIPAA regulations.
- Review of the medical records will not result in greater than minimal risk by taking appropriate precautions to protect the confidentiality of the information.

Agree ☑

3.0 ***Indicate why the potential study participants' rights and welfare would not be adversely affected by waiving consent to review their medical records.**

Check all that apply.

- ☑ Precautions will be taken on protect the confidentiality of the research participants
- ☑ The information from the medical records will not be used in any way other than to identify potential research participants
- ☐ Other

3.1 **If other, describe**

4.0 ***Indicate why the research could not practicably be carried out without a waiver of consent.**

Check all that apply.

- ☑ The identities of the potential study participants who would meet the criteria for this study would not be known without access to their medical records
- ☐ Other

4.1 **If other, specify**

5.0

NON-UC INSTUTITION(S) / AGENCY(IES) HIPAA POLICIES AND PROCEDURES

If your research will involve access, use, or disclosure of PHI held by a non-UC institution/agency, please provide your assurances that you will comply with that (those) instutition(s)/agency(ies)' HIPAA policies and procedures.

Agree ☑

ID: IRB#14-000932 View: NEW 19.1 - Eligibility Screening

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Eligibility Screening

1.0 ***Will you be conducting a preliminary assessment with potential research participants to determine study eligibility during the recruitment process?**

(•) **Yes** ( ) No

ID: IRB#14-000932 View: NEW 19.2 - Eligibility Screening - Plans

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Eligibility Screening - Plans

You indicated that eligibility screening will be conducted during the recruitment process (Section 19.1/item 1). Please provide the following information.

1.0 ***Will private identifiable information be collected during the screening?**

(•) **Yes** ( ) No

1.1 **If private identifiable information is collected during screening, are there plans to retain data from participants found to be ineligible for the study?**

( ) Yes (•) **No**

1.2 **If private identifiable data will be collected during the screening, indicate your plans for retaining the data.**

- [ ] The data will be retained with identifiers
- [ ] The data will be retained without identifiers
- [x] The data will be destroyed

1.2.1 **If you chose more than one response above, explain.**

2.0 ***Indicate your plans for obtaining informed consent and/or parental permission for the screening procedures.**

Check all that apply.

- [x] Oral consent will be obtained for the screening procedures. Participants will not be asked to sign a consent form (Waiver of written consent).
- [ ] A waiver of informed consent is requested for the screening procedures
- [ ] A waiver of Research Authorization for HIPAA is requested for the screening procedures.
- [ ] Signed consent will be obtained prior to performing any of the screening procedures

2.1 **If you checked more than one plan above, list the study groups and the plan that you will use for each.**

3.0

Describe how screening will be performed.

The subject will be reached by phone or will visit the offices of Drs. Lu and/or Denis and/or Niu to be interviewed about potentially participating in the study. If by phone, the potential subject will be asked to provide Dr. Lu and/or Denis and/or Niu with access to their chart. A thorough history and physical and chart review will be used to determine eligibility/suitability for study participation.

3.1 **Attach screening script(s), if applicable.**

Document Name	Document Version #
Phone_Screening_Script_clean_07312015.doc	0.01
Phone_Screening_Script_track_07312015.doc	0.01

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Oral Consent - For Screening Procedures

You indicated that you are obtaining oral consent for the screening procedures (Section 19.2/Item 2). Please provide the following information.

1.0 ***Indicate the reason that you are requesting to conduct an oral consent process and/or parental permission instead of obtaining signed consent.**

- ○ The research is minimal risk and does not involve any procedures for which written consent is normally required outside the research setting (e.g., in everyday life written consent is not needed for minimal risk surveys, non-invasive health measurements, etc.) (45 CFR 46.117 c2)
- ◉ The only record linking the participants and the research would be the consent document, and the main risk of research would be a breach of confidentiality (45 CFR 46.117 c1).

  e.g., Participants could suffer from social stigma, embarrassment, or other harms if it became known that they participated in research that identified them as having issues including, but not limited to, risky sexual behaviors, HIV, or mental health problems.

If you indicated that the **main risk is a breach of confidentiality**, answer 1.1 if appropriate.

1.1 **According to DHHS regulations at 45 CFR 46.117(c1) when the main risk of the research would be a breach of confidentiality and an oral consent process is used, each participant should be asked whether he/she wants documentation linking the subject with the research and the subject's wishes will govern.**

Check here if you want the IRB to consider allowing a waiver of this regulation so that you do not need to ask each subject if he/she wishes documentation.

☑ Request to waive documentation linking the participant with the research

2.0 ***Provide a description of the oral screening procedures for the study.**

Patients will be referred to Dr. Denis or Niu who will conduct a phone screening for eligibility.

ID: IRB#14-000932 View: NEW 20.1 - Informed Consent Process

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Informed Consent Process

You indicated that adults (and/or minors who are permitted to consent for themselves) are participating in the study (Section 11.2/item 1.0 or Section 12.2/item 1.0).

For additional information on minors who are permitted to consent for themselves please refer to the section "Legal Exceptions Permitting Certain Minors to Consent" in the OHRPP Guidance document, Child Assent and Permission by Parents or Guardians.

1.0 * **Indicate your plans for obtaining informed consent for this study.**

Check all that apply:

- [x] **Signed consent** will be obtained from the research participant or Legally Authorized Representative.
  - Signed consent means research participants will be asked to **sign and date** a written consent form.
- [ ] **A waiver of signed consent is requested for the entire study.** One of the following procedures will be conducted:
  - **A written information sheet will** be used. Signed consent will not be obtained from research participants.
  - **Oral consent** will be obtained from the research participant or Legally Authorized Representative (LAR)
  - This option should be selected if the study involves consenting participants via the internet.
- [ ] A **waiver of consent** is being requested.
  - Research participants will **not** be asked to sign a consent form or give oral consent
- [ ] Consent will be obtained by a collaborating institution.

1.1 **- If you checked more than one plan above, list the study groups and the plan that you will use for each.**
- If you checked "Consent will be obtained by a collaborating institution", explain the consent process and upload a copy of the most recent approved consent document in item 1.2.

1.2 **If applicable, attach the consent document(s) from collaborating institution(s).**

Document Name	Document Version #
There are no items to display	

ID: IRB#14-000932 View: NEW 20.3 - Description of the Consent Process

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking “Save” or “Continue.”

Description of the Consent Process

1.0 ***Indicate the type of setting(s) in which the consent process will be conducted.**

Check all that apply.

- [ ] In a private home
- [x] In a private room
- [ ] In a waiting room
- [ ] In a public setting
- [ ] In a group setting
- [ ] On the internet
- [ ] Over the telephone
- [ ] Other

1.1 **If you checked more than one response, or indicated other, describe.**

1.2 **If the setting is not private, describe the measures to protect confidentiality or indicate "not applicable."**

2.0 ***Indicate the measures that will be taken to provide prospective research participants with sufficient opportunity to consider whether or not to participate in the study.**

Check all that apply.

- [x] Member(s) of the study staff will meet with the prospective participants/families to review the consent document(s) and/or provide an oral explanation of the study. Individuals will be given a chance to ask questions before making a considered decision about whether or not to participate in the study.
- [ ] Prospective participants/families will have the opportunity to take the consent form(s) home and may discuss the documents with others prior to deciding whether or not to participate in the study.
- [ ] Prospective participants will self-administer the consent and send it back if they decide to participate in the study.
- [ ] Other

2.1 **If you indicated other, describe.**

3.0 ***Indicate the length of time subjects are given to decide whether they wish to participate in the study.**
48 hours

4.0 ***How will you assess whether subjects understand the information conveyed during the consent process?**

Check all that apply.

- [ ] Use the Subject Comprehension Tool form for research
- [x] Investigator or study team member will evaluate during the consent process
- [ ] Other
- [ ] Not Applicable

4.1 **If you indicated other, describe.**

5.0 ***Attach copies of the informed consent documents, information sheets, consent scripts as applicable to this study. Include copies of translated forms, if applicable.**

Document Name	Document Version #
DoD Bladder Consent Form Clean 5-28-15.docx	0.01

ID: IRB#14-000932 View: NEW 22.1 - Cultural Considerations

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Cultural Considerations

The following items are designed to acquaint the IRB with cultural features of the population that you are studying that may require procedures to ensure truly informed consent.

1.0 ***Check all that apply to the population(s) with which this study will be conducted.**

- [ ] Participants may be illiterate or insufficiently literate to be able to comprehend a conventional written informed consent form.
- [ ] The participants may be reluctant or unwilling to sign a written informed consent form.
- [ ] The husbands make decisions for their wives.
- [ ] Elders make decisions for younger adult family members.
- [ ] Elders make decisions for their community.
- [ ] It is considered impolite to refuse a request.
- [ ] People are fearful of refusing requests that they regard as coming from authorities.
- [x] **None of the above are applicable to this study.**

1.1 **If any of the above items are applicable to this study, indicate the steps that you will take to ensure voluntary participation after providing the study information, and if applicable, any planned involvement with the community regarding the consent process.**

ID: IRB#14-000932 View: NEW 22.2 - Non-English Speaking Study Participants

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Non-English Speaking Study Participants

You indicated that you would involve non-English speaking participants in the study (Section 11.2/Item 2.0) and/or that there is a possibility that non-English speaking participants may be enrolled in the study (Section 11.2/Item 3.0). Please provide the following information.

1.0 ***Indicate the method that you use to conduct the consent process[1] with participants who do not speak English.**

Check all that apply.

- ☐ The consent form and other study documents will be available in the participants' primary language. Study personnel (or qualified translators) able to discuss the participation in the patients' language will be present for the consent process.
- ☑ Study staff or qualified translators will discuss the study in the participants' language.
- ☐ An oral consent process will be used. Study personnel (or qualified translators) able to discuss the participation in the participants' language will be present for the consent process.
- ☐ The short form or another method will be used to conduct the consent process.

Important Note: The short form may be used in very limited circumstances. For additional information please refer to the " 'Short Form' Method" section of the OHRPP guidance document, Research Involving Non-English Speaking Research Participants.

1.1 **If you checked "short form or another method", provide additional details.**

2.0 ***How will you maintain the ability to communicate with non-English speakers throughout their participation in the study?**
Indicate "N/A" if not applicable to your study.
Members of the research staff a fluent in several languages.

3.0 ***If you are conducting research for which there is a real or foreseeable risk of biomedical harm in the state of California, indicate your agreement that you will provide the participants who do not read, speak, or understand English a copy of the Research Participants Bill of Rights in a language in which they are fluent. Translations into the most common languages in the greater Los Angeles area are available for download on the OHRPP website.**

- ◉ Agree
- ○ Not Applicable

[1] *If minors are involved in the study, this would also include the processes of obtaining parental permission and assent, as applicable.*

ID: IRB#14-000932 View: NEW 23.1 - Department of Defense

Warning: Save your work at least every 15 minutes by clicking “Save” or “Continue.”

Department of Defense

You indicated that this study is being supported and or conducted in collaboration with the Department of Defense (Section 6.1/item 2.0). Please provide the following information.

1.0 ***How is your project linked to the Department of Defense (DOD)?**

Check all that apply.

- [x] The project is funded by the DOD
- [ ] The project involves cooperation or collaboration with DOD
- [ ] The project uses DOD property, facilities, or assets
- [ ] DOD personnel (military or civilian) will be research participants

2.0 ***Will surveys or interviews be conducted with DOD personnel as part of this study?**

○ Yes ◉ **No**

2.1 **If yes, consult with your program officer to identify the survey requirements of the applicable branch of the DOD.**

- [ ] Survey approval is not required
- [ ] Documentation of Survey approval is attached below
- [ ] UCLA IRB approval is required prior to approval from DOD
- [ ] Other

2.1.1 **If you indicated "Other," specify.**

2.2 **Attach documentation of DOD survey approval (if applicable).**

Document Name	Document Version #
There are no items to display	

3.0 **Prohibitions or limitations related to DOD research**

Review and indicate your assurance that you will comply with the following limitations.

3.1 ***Captured or Detained Persons**

SECNAVINST 3900.39D (Section 6(a)(8)) prohibits research involving "any person captured, detained, held, or otherwise under the control of DoD personnel (military or civilian, or contractor employee)" except DoD personnel held for law enforcement purposes.

Agree [x]

3.2 ***Payment to Active Duty Personnel**

Based on 24 USC 30, the military limits research payments for Active Duty personnel. Unless on leave status during participation, such personnel may not receive payment for participation except for blood donation. Payment for blood donation may not exceed $50 per blood draw.

Agree [x]

3.3 ***Classified (or Sensitive but Unclassified) Research**

Because classified research involves restriction of the dissemination of results, UCLA institutional practice is to not accept such research. This prohibition includes the designation of "sensitive but not classified."

Agree [x]

ID: IRB#14-000932 View: NEW 23.2 - DOD - Study Greater than Minimal Risk

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

DOD - Study Greater than Minimal Risk

You have indicated that this study is greater than minimal risk (Section 5.1/item 1.0).The following information is required by the DOD.

1.0 **Research Monitor.** The following information is required regarding designation of a research monitor for this study.

1.1 ***Attach a copy of the Research Monitor's *curriculum vitae.***

Document Name	Document Version #
CV - Victor Chang.doc	0.01

1.2 ***Attach a copy of the letter from the Research Monitor accepting the role.**

Document Name	Document Version #
20140930083527367.pdf	0.01

1.3 ***Indicate where the Research Monitor is named and his/her role is described.**
Check all that apply.

- [ ] Privacy and Confidentiality section of the consent form(s) (required only if the Monitor will have access to individually identifiable data)
- [x] In Section 15 (Data & Safety Monitoring) of this application
- [ ] In the attached protocol for this study

1.3.1 **If you indicated that the Research Monitor's role is described in the attached protocol, indicate the page number and/or section where the information can be found.**

2.0 ***Protections for Military Personnel. Check the assurance(s) applicable to your recruitment plan**

- [ ] Not Applicable - Department of Defense personnel (military or civilian) are not a target population
- [x] I will ascertain that an individual's decision about participation has not been influenced by unit officers or senior noncommissioned officers (NCOs)
- [ ] I will exclude unit officers and senior NCOs from recruitment/consent sessions for units under their command
- [ ] I will offer separate recruitment/consent sessions for officers and NCOs excluded from sessions held for their units
- [ ] An ombudsperson not connected to the research or to the unit shall be present to monitor group recruitment briefings
- [ ] Other - I am implementing the following protections not specified above

2.1 **If you indicated "other," describe.**

Additional Information and/or Attachments

1.0 **Attach any other documents that have not been specifically requested in previous items, but are needed for IRB Review.**

Document Name	Document Version #
Alaynick_CV_Long_2014.pdf	0.01
CV_Morteza Modaber.pdf	0.01
CV_Paymon_Rezaii.pdf	0.01
DCL BIosketch.pdf	0.01
PersonnelCITIPackages.pdf	0.01

2.0 **If there is any additional information that you want to communicate about this study, include it in the area provided. Note: this section should not be used instead of the standard application items.**

ID: IRB#14-000932 View: NEW 100.0 - Instructions for Study Submission

Instructions for Study Submission

You have completed your application, **but it has *not yet been submitted*.**

FOLLOW THESE STEPS TO SUBMIT THE APPLICATION TO THE IRB FOR REVIEW:

1. Click the **Finish** button to return to exit the SmartForm and return to the study workspace.
2. Use the **View SmartForm Progress** function to make sure that the application is complete.
3. If you are the PI or PI Proxy, click **Submit Study** under **My Activities**. If you are a member of the study team, you can let the PI know that the study is ready to submit by clicking **Send Ready Notification.**
4. Once the study is submitted, the state indicator at the top of the page will no longer display **Pre-Submission.**
5. After submission of the study, the **PI Assurances** activity will immediately become available under **My Activities**. The PI should provide his/her assurances at that time. If the PI is not available, the study can be submitted by a PI Proxy and the assurances provided at a later time. The study will be reviewed by the IRB while the **PI Assurances** are pending; however, it will not be approved until the **PI assurances** are completed.
6. ***If there is a Faculty Sponsor for the study***: The study can not be submitted to the IRB until the Faculty Sponsor provides his/her assurances through **FS Assurances** activity.

ID: IRB#14-000932 View: Display - Method Description

Audio, Visual or Digital Recordings

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Behavioral Observations (only applicable if you selected Exempt Category 2 in section 5.3)

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Certificate of Confidentiality

Certificates of Confidentiality are issued by the National Institutes of Health (NIH) to protect the privacy of research subjects by protecting investigators and institutions from being compelled to release information that could be used to identify subjects with a research project. Certificates of Confidentiality are issued to institutions or universities where the research is conducted. They allow the investigator and others who have access to research records to refuse to disclose identifying information in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. The project does not need to be funded by NIH to obtain a Certificate of Confidentiality. For additional information see http://grants.nih.gov/grants/policy/coc/

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Clinical Trial of a Drug, Biologic, Device or a Behavioral Intervention

A clinical trial is a research study designed to answer specific questions about medical or behavioral treatments. The trial may be interventional or observational. Interventional studies are those in which the research participants are assigned by the investigator to a treatment or other intervention, and the outcomes measured. Observational studies are those in which individuals are observed and the outcomes are measured by the investigators.

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Community Based Research

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Controlled Substances (Schedule I or II)

Check here only if you are using a Schedule I or II Controlled substance in this study. Research using Schedule I or Schedule II controlled Substances must be submitted to the Research Advisory Panel of California for review and approval prior to initiation. Research using Schedule III, IV, or V Controlled Substances as a study drug do not require review by the Research Advisory Panel. For further information see: http://ag.ca.gov /research/guide.php o Schedule I Controlled Substances are drugs or substances with a high potential for abuse, that have no currently accepted medical use in treatment in the United States. Examples of Schedule I Controlled Substances are: heroin, lysergic acid diethylamide (LSD), methylenedioxy-methamphetamine (MDMA), marijuana, and psilocybin. o Schedule II Controlled Substances are drugs or substances with a high potential for abuse, that have a currently accepted medical use in treatment in the United States, or a currently accepted medical use with severe restrictions. Examples of Schedule II Controlled Substances are: fentanyl, methadone, methylphenidate, morphine, and oxycodone. For further information see: http://www.deadiversion.usdoj.gov/schedules/index.html

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Deception or Partial Disclosure

Deception includes withholding information about the real purpose of the study or purposely giving subjects false information about some aspect of the research to prevent bias. Some professions, such as the American Psychological Association (APA) have ethical codes regarding the use of

deception in research. (See sections 8.07 and 8.08 at http://www.apa.org/ethics/code/index.aspx#807) If deception is included in the study, you must also apply for approval of a waiver of the informed consent process (Section 20.1) in addition to selecting the other consent procedures planned for the study (e.g., written or oral consent).

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Devices/Diagnostics (including Humanitarian Devices - HUD)

A medical device is defined, in part, as any health care product that does not achieve its primary intended purposes by chemical action or by being metabolized. Medical devices include, among other things, surgical lasers, wheelchairs, sutures, pacemakers, vascular grafts, intraocular lenses, and orthopedic pins. Medical devices also include diagnostic aids such as reagents and test kits for in vitro diagnosis (IVD) of disease and other medical conditions such as pregnancy. For further information see: http://www.fda.gov/oc/ohrt/irbs/irbreview.pdf

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Drugs/Biologics/Dietary Supplements

- Drug: The term "drug" means: articles recognized in the official United States Pharmacopoeia, official Homoeopathic Pharmacopoeia of the United States, or official National Formulary, or any supplement to any of them; and articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals; and articles (other than food) intended to affect the structure or any function of the body of man or other animals.
- Biologics vs. Drugs: Most drugs consist of pure chemical substances and their structures are known. Most biologics, however, are complex mixtures that are not easily identified or characterized. Biological products differ from conventional drugs in that they tend to be heat-sensitive and susceptible to microbial contamination. This requires sterile processes to be applied from initial manufacturing steps. For more information see: http://www.fda.gov/consumer/updates/biologics062608.html#drugs
- Dietary Supplements are products that are intended to supplement the diet and have one of the following ingredients:
  - A vitamin
  - A mineral
  - An herb or other botanical
  - An amino acid
  - A dietary substance for use by man to supplement the diet by increasing the total daily intake
  - A concentrate, metabolite, constituents, or an extract of combinations of these ingredients.

For additional information see: http://www.foodsafety.gov/~dms/supplmnt.html

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Expanded Access to Drug, Device or Biologic for Treatment Purposes (aka Compassionate Use, Treatment Use)

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Genetic Analyses/Genotyping

Genetic analyses/genotyping include, but are not limited to, studies of inheritable conditions or traits, gene markers or mutations, and pedigrees.

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Human Embryonic Stem Cells and/or Induced Pluripotent Stem Cells

Research with human embryonic stem cells (hESC) and related lines requires IRB review under the following conditions: o Clinical research in which human subjects are given hESCs or related products. o When the UCLA research team will have a research related direct interaction or intervention with the cell donors, including donation of blastocysts or gametes for the purpose of creating hESCs,. o Cells provided to the UCLA research team that have identifiers or codes that can be linked back to the donor. Research involving hESC requires review and approval by the ESCRO Committee. For further information see: http://www.stemcell.ucla.edu/research

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Human Gene Transfer/ Recombinant DNA

Studies involving gene transfer and/or recombinant DNA require approval of the UCLA Institutional Biosafety Committee (IBC) and the NIH Recombinant DNA Advisory Committee (RAC) . Human gene transfer is an investigational method for correcting defective genes responsible for disease development through one of the following techniques: o A normal gene may be inserted into a nonspecific location within the genome to replace a nonfunctional gene. o An abnormal gene could be swapped for a normal gene. o The abnormal gene could be repaired through selective reverse mutation, which returns the gene to its normal function. o The regulation of a particular gene could be altered. Recombinant DNA molecules, according to the NIH Guidelines, are defined as either: (i) molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above.

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Infectious Agents

Studies involving the use of Risk Group 2 or 3 infectious agents (such as bacteria, fungi, parasites, prions, rickettsia, viruses, etc.) require approval of the UCLA Institutional Biosafety Committee (IBC).

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Non-FDA approved medical equipment used with UCLA hospital patients or research participants that operate under the UCLA Hospital License.

Clinical Engineering is responsible for completing incoming inspections on investigational devices that are used to diagnose, treat or monitor a patient and that are used in the patient care area on site at UCLA, but *not* in other hospitals such as Cedars Sinai, CHLA, or Drew. If a device is FDA and/or testing - laboratory approved for the purpose it was designed, then evaluation is not required of the device. If you have a copy of an inspection report from Clinical Engineering, please attach here. As appropriate, please contact Clinical Engineering at 310-267-9000 to arrange an inspection.

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Radiation (Standard of Care or Investigational use of radioactive materials or ionizing radiation)

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Substance Abuse Research (with Medication)

Research for the treatment of controlled substance addiction or abuse that uses any drug (scheduled or not) as treatment, requires the review and approval of the Research Advisory Panel of California prior to initiation. For further information see: For further information see: http://ag.ca.gov /research/guide.php

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Treatment in an Emergency Setting (with request to waive consent)

Federal regulations allow certain research activities to be conducted in emergency settings with waiver of informed consent - in the interest of facilitating potentially life-saving and life-enhancing research with protecting the rights and welfare of participants. For further information see: o OHRP Guidance: http://www.hhs.gov/ohrp/humansubjects/guidance/hsdc97-01.htm o FDA Guidance: http://www.fda.gov/oc/ohrt/irbs/except.html

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

None of the above

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

frontiers in Molecular Neuroscience

REVIEW
published: 16 June 2015
doi: 10.3389/fnmol.2015.00025

Molecular and cellular development of spinal cord locomotor circuitry

*Daniel C. Lu, Tianyi Niu and William A. Alaynick**

Department of Neurosurgery, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, USA

The spinal cord of vertebrate animals is comprised of intrinsic circuits that are capable of sensing the environment and generating complex motor behaviors. There are two major perspectives for understanding the biology of this complicated structure. The first approaches the spinal cord from the point of view of function and is based on classic and ongoing research in electrophysiology, adult behavior, and spinal cord injury. The second view considers the spinal cord from a developmental perspective and is founded mostly on gene expression and gain-of-function and loss-of-function genetic experiments. Together these studies have uncovered functional classes of neurons and their lineage relationships. In this review, we summarize our knowledge of developmental classes, with an eye toward understanding the functional roles of each group.

Keywords: interneuron, motor neuron, transcription factor, locomotion, sensory, circuit

OPEN ACCESS

Edited by:
Robert W. Burgess, The Jackson Laboratory, USA

Reviewed by:
Bruce R. Johnson, Cornell University, USA
Francisco Javier Alvarez, Emory University, USA

****Correspondence:***
William A. Alaynick, Department of Neurosurgery, David Geffen School of Medicine, University of California, Los Angeles, 650 Charles East Young Drive, Wasserman Building, Room 562, Los Angeles, CA 90095, USA
walaynick@mednet.ucla.edu

Received: *02 April 2015*
Accepted: *30 May 2015*
Published: *16 June 2015*

Citation:
Lu DC, Niu T and Alaynick WA (2015) Molecular and cellular development of spinal cord locomotor circuitry. Front. Mol. Neurosci. 8:25. doi: 10.3389/fnmol.2015.00025

Introduction

More than 20 distinct embryonic classes of neurons have been described in the spinal cord, and the developmental sources of their diversity have been elucidated over the past decade (**Figure 1**). This cellular diversity has been organized into a schema that defines major groups of neurons based on their expression of embryonic transcription factors. The major characteristics of these classes their generation, transcription factors, subsets, positions, neurotransmitters, connections, and functions are summarized here.

Spinal cord development is subject to phylogenetically ancient organizing principles such as those that guide segmentation from the invertebrates, such as arthropods, to the vertebrates, such as mammals. Cellular identities in vertebrate spinal cord are specified during development along the three basic spatial axes of the embryonic body plan – rostral–caudal, dorsal–ventral, and medial–lateral. In addition, there is a temporal influence of development on these spatial coordinates such that distinct cell fates emerge at different times during development. This yields a four dimensional system for establishing spinal neuron cell fate that has been reviewed extensively (Jessell, 2000; Jankowska, 2001; Lee and Pfaff, 2001; Muroyama et al., 2002; Helms and Johnson, 2003; Goulding and Pfaff, 2005; Kiehn, 2006; Ladle et al., 2007; Stepien and Arber, 2008; Dasen and Jessell, 2009; Goulding, 2009; Grillner and Jessell, 2009; Hegarty et al., 2013).

To summarize briefly, the rostral–caudal positional identities are coordinated by opposing gradients of fibroblast growth factor (Fgf, caudalizing) and retinoic acid (RA, rostralizing; **Figure 2**; Muhr et al., 1999; Liu et al., 2001; Dasen et al., 2008). The dorsal–ventral axis is governed by ventralizing Sonic hedgehog (Shh) produced by the floorplate, and dorsalizing signals from the roof plate such as bone morphogenetic proteins (BMPs) and Wnts (which are members of the Wingless + MMTV integrants, *Int* family). These diffusible morphogens form gradients that activate specific transcriptional responses at defined points in the gradient (Roelink et al., 1994; Liem et al., 1995;

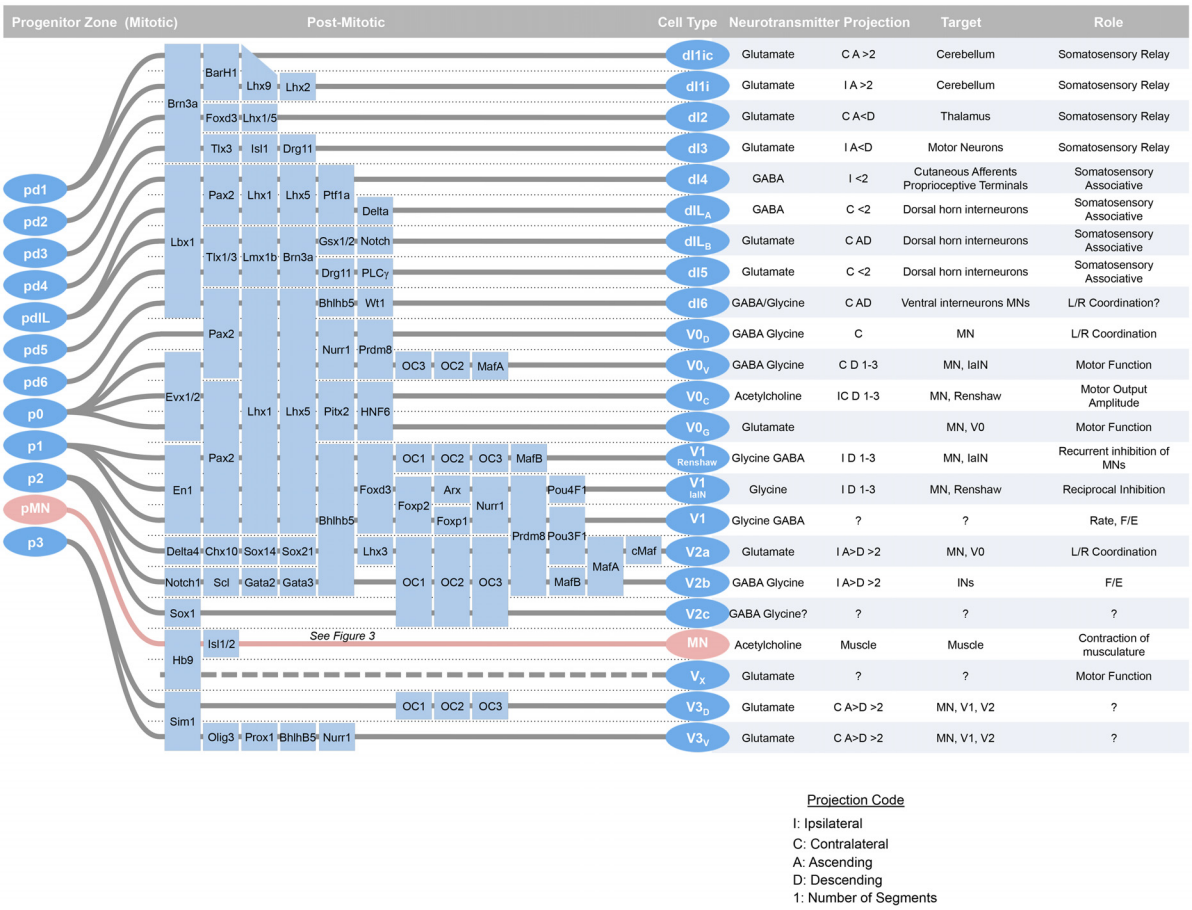


FIGURE 1 | At around mid-gestation, progenitors exit the cell cycle and begin to take up characteristic setting positions, extend axons, and express transcription factors and neurotransmitter biosynthetic enzymes. Over the last week of development, 23 classes of neurons can be defined by transcription factor expression. Adapted from Alaynick et al. (2011).

Ericson et al., 1996; Lee et al., 1998; Megason and McMahon, 2002; Muroyama et al., 2002; Timmer et al., 2002). These transcriptional programs first specify and reinforce the identities of progenitor cells, and second, act to oppose adjacent transcriptional programs and sharpen boundaries between progenitor zones. In the ventral cord, these transcription factors are grouped into two classes, those that are inhibited by Shh (Class I) and those that are activated by Shh (Class II; Briscoe et al., 2000). Spinal cord development is also organized along a medial–lateral axis dividing progenitor cells that are located adjacent to the lumen of the neural tube, medially, whereas differentiating progeny migrate laterally. Over time, a given progenitor domain defined by these spatial coordinates may sequentially produce distinct cellular classes.

Within an idealized spinal cord segment, this system establishes thirteen progenitor pools along the dorsal–ventral axis (**Figure 2**). There are eight dorsal interneuron progenitor divisions, pd1–6 and the late-born pdILA and pdILB, four ventral interneuron progenitor divisions, p0–3, and one motor neuron progenitor domain, pMN (Alaynick et al., 2011). The identities of these domains are predominantly defined by basic-helix-loop-helix (bHLH) domain transcription factors, such as Ngn, Olig2, and Math (Bermingham et al., 2001; Gowan et al., 2001; Novitch et al., 2001; Scardigli et al., 2001; and homeodomain proteins, such as Pax3, Dbx1, and Nkx6.1 (Briscoe et al., 2000; Vallstedt et al., 2001; Subsequently, additional transcription factors, predominantly of the LIM-homeodomain family, such as Lhx1 and Isl1, are expressed in sub-groups of these domains, further refining cell fate into at least 23 distinct classes (Tsuchida et al., 1994; Gross et al., 2002; Muller et al., 2002; Thaler et al., 2002; Cheng et al., 2004).

Ventral Compartment of Distinct Progenitor Cells

pMN Fate

The pMN cell domain gives rise to: (1) 100s of genetically distinct groups of cholinergic alpha motor neurons clustered into motor pools that innervate specific skeletal muscles; (2) gamma motor neurons that innervate intrafusal fibers of specific skeletal

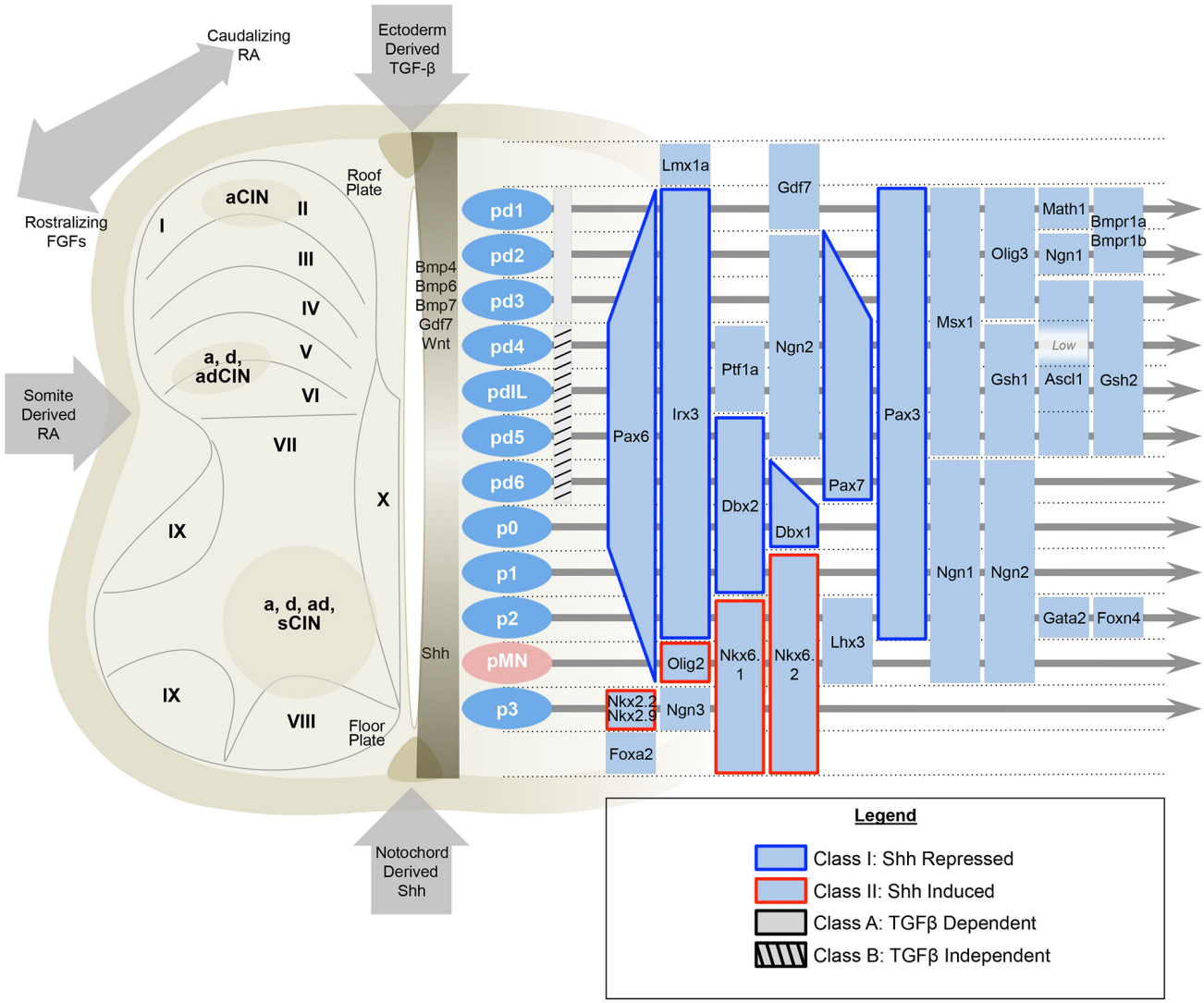


FIGURE 2 | The early spinal cord (e9.5–e11) is influenced by Sonic-hedgehog (Shh) ventrally, ectoderm-derived TGF-beta family members dorsally, and retinoic acid from the somite, laterally. This establishes 13 progenitor domains (including the late born pdILA and pdILB) that express transcription factors that help to define progenitor identities and refine boundaries between progenitor domains. Ventrally, Class I transcription factors are repressed by Shh (e.g., *Irx3*), while Class II are induced (e.g., *Olig2*). Similarly, the dorsal-most domains, pd1–pd3, are dependent on TGF-beta and the pd4–pd6 and pdIL domains are independent of TGF-beta signaling. Adapted from Alaynick et al. (2011).

muscles for proprioception; (3) the predominantly thoracic (T1–12, and to L1 and L2 in some species) cholinergic preganglionic sympathetic neurons; (4) the cholinergic parasympathetic motor neurons in the sacral (S2–4) cord; and (5) oligodendrocytes found throughout the spinal cord (**Figure 3**). The motor neurons that effect muscle movement are primarily alpha, with fewer beta, motor neurons.

pMN Birth and Early Development

The recognition that a supernumerary notochord could induce the generation of additional motor neurons led to the identification of the diffusible morphogen, Shh, that induces neural precursors to a MN fate (Watterson, 1965; Roelink et al., 1994). An early marker of motor neuron development, the LIM-HD transcription factor, *Isl1*, indicated that motor neuron precursors are born between HH Stages 15 and 17 in chick and beginning at E9.5 (~25 somites) in mouse (**Figure 4A**; Ericson et al., 1992; Roelink et al., 1994; Pfaff et al., 1996; Gould et al., 2006). These progenitors will give rise to somatic alpha motor neurons that innervate skeletal muscle in the medial and lateral motor columns (LMCs), gamma motor neurons that innervate intrafusal fibers of the muscle spindles, and preganglionic motor neurons of the autonomic nervous system. Generation of each of these classes and their organization

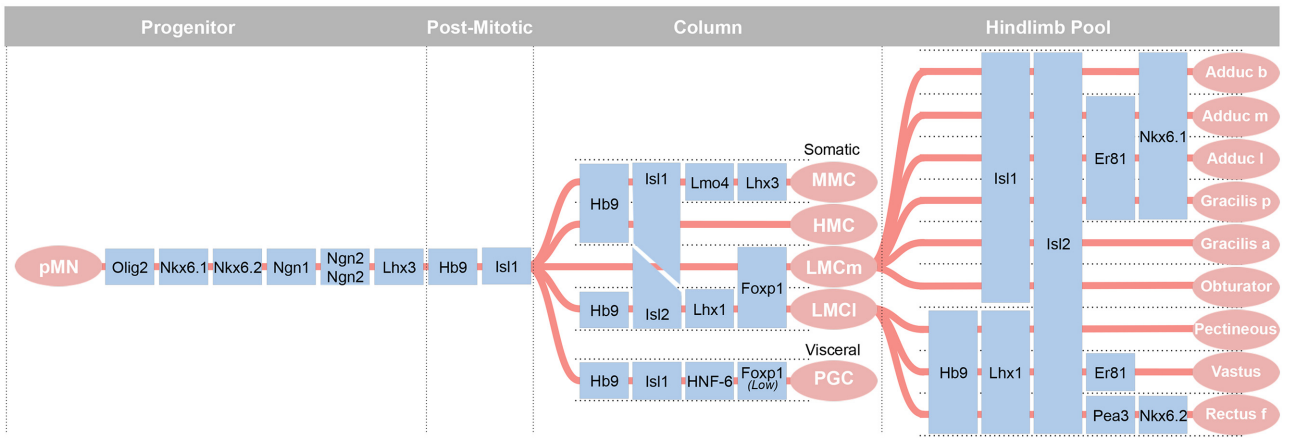


FIGURE 3 | The most diverse spinal cord neuron class belongs to the motor neurons. These arise from a uniform progenitor domain before differentiating in to classes that can be grouped by columns and by motor pools. Motor pools are clusters of motor neurons that innervate a single muscle. A transcription factor code is emerging to define each of the over 200 motor pools that innervate distinct muscles. Adapted from Alaynick et al. (2011).

into motor columns and motor pools requires the subsequent expression of additional transcription factors (Lin et al., 1998; Dasen et al., 2005, 2008). These factors then drive the unique characteristics of that motor pool, such as guidance to the target and establishment of proper connectivity with sensory neurons and interneurons. Interestingly, this transcriptionally defined program is complemented by activity-dependent processes that control cellular connectivity and function (Hanson and Landmesser, 2004; Myers et al., 2005). In the case of gamma motor neurons, the nuclear receptor *Errγ* is expressed in these motor neurons and their survival is dependent on GDNF signaling (Gould et al., 2008; Friese et al., 2009; Shneider et al., 2009; Ashrafi et al., 2012).

The motor neuron progenitor domain is ventral to the *Irx3* expressing p2 domain that delimits *Olig2* expression and is dorsal to the p3 domain that expresses *Nkx2.2* and *Nkx2.9* to delimit *Pax6* expression. The expression of *Nkx6.1* and *Nkx6.2* acts to limit transcription factor expression to *Olig2,* that in turn drives the expression of MN transcription factors *Hb9 (Mnr2 in chick)*, and *Ngn2* (Briscoe et al., 2000; Sander et al., 2000; Vallstedt et al., 2001; Shirasaki and Pfaff, 2002). *Hb9*, expressed during the final cell division of pMNs, is sufficient to drive the expression of *Isl1*, *Isl2*, *Lhx3*, and *ChAT*—as well as its own expression—establishing pMN independence from Shh (Tanabe et al., 1998). Like *Mnr2*, the HD transcription factor, *Hb9*, can induce the formation of motor neurons when ectopically expressed. Loss of *Hb9* in mouse, however, results in ectopic upregulation of a V2 IN marker gene, *Chx10*, but does not result in complete loss of motor neurons or of fictive locomotion (Arber et al., 1999; Thaler et al., 1999; Alaynick, Pfaff unpublished observations).

Motor Neuron Subtypes

The Medial and Hypaxial Motor Columns (MMC and HMC)

The medial sub-group of motor neurons innervates axial musculature and is found the length of the spinal cord. There are two divisions of this group, the medial motor column (MMC) and the hypaxial motor column (HMC or MMCl). Both express *Isl1* and *Isl2*, although the ratio of expression varies, with greater *Isl1* expression in the HMC than MMC at E11.5 and greater *Isl2* in the MMC than HMC by E13.5 in mouse (Tsuchida et al., 1994; Thaler et al., 2004). The MMC innervates dorsal or epaxial musculature, while HMC innervates ventral or hypaxial musculature. Initially all motor neuron progenitors express the LIM homeodomain transcription factor, *Lhx3*. *Lhx3* expression is maintained in the MMC while *Lhx3* expression is downregulated in the HMC and LMC (Tsuchida et al., 1994). Motor neuron (*Hb9* promoter) dependent expression of *Lhx3* results in conversion of LMC motor neurons to a MMC identity (Sharma et al., 2000).

The Lateral Motor Column (LMC)

At limb levels, the 50 or so muscles of the limb are innervated by motor neurons occupying a lateral motor column (Landmesser, 1978). Neurons of the lateral portion of the LMC (LMCl) are later born than the MMC motor neurons, and like the cortex, migrate in an inside-out arrangement such that LMC neurons are born in the proliferative ventricular zone of the pMN domain and then migrate through the MMC to form the LMC. While initially expressing *Lhx3*, a hallmark of MMC identity, these motor neurons down-regulate *Lhx3* by an unknown mechanism and begin to express transcription factors not found in MMC that are definitive for LMC identity. The factors include *Foxp1*, *Lim1*, and the enzyme *Raldh2* (Sharma et al., 1998, 2000; Sockanathan and Jessell, 1998). The lateral motor column has lateral (LMCl) and medial (LMCm) divisions that innervate the dorsal and ventral portions of the limb, respectively, and these cell fates are partially regulated by RA signaling (Sockanathan et al., 2003; Ji et al., 2006). In the LMCl, *Lim1* and *Hb9* are expressed while *Isl1* is downregulated. In the LMCm, there is low *Hb9* and maintained *Isl1* expression. The LMCm and LMCl both express *Isl2*, which is downregulated in the MMC and HMC (Misra et al., 2009). The LMCm and LMCl are further subdivided into motor pools, each

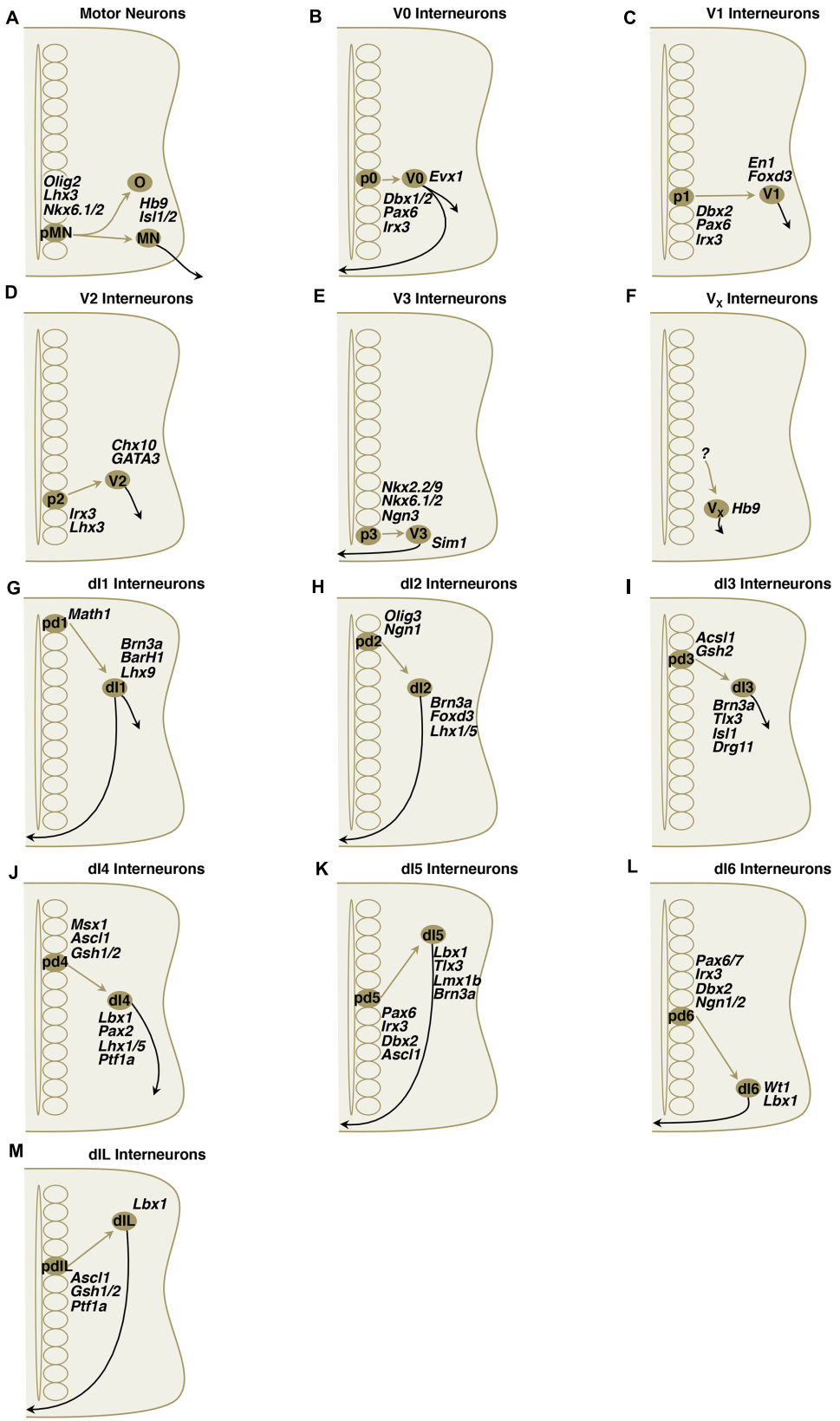


FIGURE 4 | (A–M) Simplified schematic illustrations of development of MNs and ventral/dorsal subclass interneurons with important transcriptional factors.

innervating a specific muscle of the limb. These individual motor pools are defined by their expression of *Ets* and *Nkx* transcription factors that constitute a more refined transcriptional code (De Marco Garcia and Jessell, 2008).

The rostro-caudal regions of the LMC appear to be determined in part by homeobox (Hox) genes. *Hox6* is characteristic of brachial level, *Hox9* of thoracic and *Hox10* of lumbar. Disruption of the Hox genes in mouse or chick has shown that these boundaries can be profoundly altered to create an expansion of lateral motor columns into thoracic regions (Jung et al., 2010). More strikingly, loss of the *Hox* co-factor *Foxp1* disrupts the ability of motor neurons to incorporate the homeobox code for spatial information, and results in a loss of defined motor pools in the LMC (Dasen et al., 2008).

Preganglionic Motor Neurons (PGC)

Preganglionic motor neurons of the sympathetic nervous system are the most dorsal motor neurons and can be identified by their expression of *ChAT*, *NADPH Diaphorase*, and some members of the one-cut transcription factor class (Francius and Clotman, 2010). Preganglionic motor neurons are also dependent on the downregulation of *Lhx3* and are lost with continued, *Hb9*-dependent expression of *Lhx3* in all motor neurons, or loss of *Foxp1 or Isl2* (Sharma et al., 2000; Thaler et al., 2004; Dasen et al., 2005, 2008).

Spinal Interneurons

A great deal has been learned about the development of discrete classes of interneurons by describing them by electrophysiology, behavioral output, and by expression of proteins involved in transcription, neurotransmitter signaling, and intracellular signaling. Currently, this schema has defined over 20 interneuron types in the spinal cord. While one can argue that every neuron has a unique molecular/genetic expression profile, dendritic arborization and axonal projection pattern, this grouping schema has been useful in organizing interneurons into functionally related groups.

Historically, two broad groups have been defined: the "V" interneurons with progenitors that are found in the *v*entral cord and are grossly associated with motor function, and a *d*orsal *I*nterneuron, dI class, associated predominantly with sensory processing. Most studies have examined development within a single or a few segments. A recent study examined rostro-caudal differences at one time point, e12.5 (Francius et al., 2013). This showed that subclasses of ventral interneurons (V0, V1, V2, and V3) exhibit distinct organizational patterns at brachial, thoracic and lumbar levels of the developing spinal cord. Furthermore, each cardinal "V"' class of ventral interneurons can be subdivided into several subsets according to further combinatorial expression of transcription factors (Francius et al., 2013). Given these caveats that likely apply to other interneuron classes, the V and dI interneuron classifications are a simplification with exceptions, some of which are listed below. Despite these limitations, the V and dI schema is a useful approach to the subject.

V0 Interneuron Characteristics

Local projecting V0 neurons are a population of primarily contralateral, with some ipsilateral projecting neurons with inhibitory or excitatory identity that send axons 2–4 spinal segments rostrally (Moran-Rivard et al., 2001; Pierani et al., 2001). They receive inputs from ipsilaterally projecting Chx10$^+$ glutamatergic V2a interneurons (Crone et al., 2008; **Figure 5**). They are the dorsal-most ventral progenitor pool and are characterized by their expression of the *Dbx* (developing brain homeobox) homeodomain transcription factor, *Evx1/2* (even-skipped homeobox 1; **Figure 4B**). *Dbx1* and *Dbx2* are expressed in dividing cells, although *Dbx1* may be briefly expressed in post-mitotic cells (see V1 discussion Pierani et al., 1999). Four V0 interneuron subclasses have been described to date: $V0_V$, $V0_D$, $V0_C$, and $V0_G$ (Pierani et al., 1999, 2001; Moran-Rivard et al., 2001; Lanuza et al., 2004; Zagoraiou et al., 2009). Early studies addressed the V0 class by eliminating *Dbx1* and showing that the *Evx1*$^+$ $V0_V$ subclass was lost because these neurons become fated to an *En1*$^+$ V1-like subclass and astrocytes (Pierani et al., 2001; Lanuza et al., 2004). Because *Dbx1* is transiently expressed, a *Dbx1*^{LacZ} knock-in allele was used to show that with loss of *Dbx1*, E18.5 embryos retained 40% of the βgal$^+$ cells and resulted in a 25% expansion in the number of *Lbx1*$^+$ *Pax2*$^+$ dl6-like commissural neurons (Lanuza et al., 2004). By perinatal time points, genetic strategies to track *Dbx1*$^+$ cells using βgal find that most of these cells are neural by expression of *NeuN* and are found in lamina VIII where commissural interneurons reside. Lineage labeling of Dbx1-derived cell reveals a large abundance of glia (Lanuza et al., 2004). Moreover, the Dbx1 lineage includes many dorsal horn neurons as this transcription factor is also expressed in dorsal domains. Loss of *Dbx1* results in loss of $V0_D$ and $V0_V$ subclasses, whereas loss of *Evx1* results in a loss of only the $V0_V$ subclass (Moran-Rivard et al., 2001; Pierani et al., 2001; Lanuza et al., 2004). V0 and V1 classes both express *Lhx1* and *Lhx5*, markers of inhibitory spinal interneurons (Pillai et al., 2007).

V0 Birth and Early Development

In mouse, the majority of *Dbx1*$^+$ progenitors appear between E10 and E13 and give rise to $V0_D$ and $V0_V$ commissural interneurons (Moran-Rivard et al., 2001; Pierani et al., 2001; Lanuza et al., 2004). *Dbx1/2* expression is found in the rostral CNS at stage 13 in chick and more caudally by stage 15 (Pierani et al., 1999). *Evx1/2* positive V0 cells are generated at stages 17 and 18 and appear in the ventral domain of *Dbx1* and *Dbx2* expression (Pierani et al., 1999). Ventral *Evx1/2* expressing V0 neurons appear at stages 17–18 within the ventral expression domain of *Dbx1* and *Dbx2*, and then migrate ventrally (Pierani et al., 1999). The V0 class appears from a *Pax6*$^+$, *Dbx1/2*$^+$, *Pax3/7*$^-$ domain that is the dorsal-most ventral progenitor domain (Pierani et al., 1999).

V0 Interneuron Subtypes

$V0_V$

The primarily inhibitory $V0_V$ class is distinguished by transient expression of the homeodomain transcription factor, *Evx1*. These cells arise from the ventral portion of the *Dbx1*$^+$ progenitor domain, and like all post-mitotic cells arising from *Dbx1*$^+$

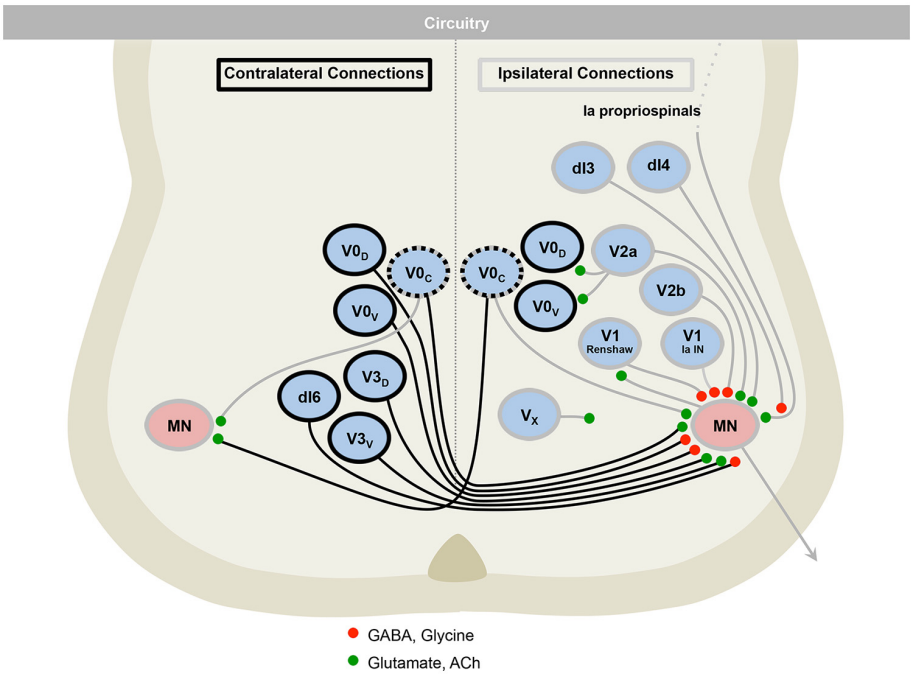


FIGURE 5 | The motor circuitry is shown in diagrammatic form in the lower panel. Here, neurons can be divided by projection patterns, that are ipsilateral, contralateral, or both. Three classes of neurotransmitter are found in the cord: excitatory glutamatergic (e.g., V2a), inhibitory GABAergic/glycinergic (e.g., V2b), and excitatory cholinergic neurons (e.g., motor neurons). Roles for neurons in defining rate (e.g., V1), left–right alternation (e.g., V0) and rhythmicity (e.g., V3), are emerging. Adapted from Alaynick et al. (2011).

progenitors, they share a similar post-mitotic migration and commissural axon pattern (Moran-Rivard et al., 2001; Pierani et al., 2001). The $V0_V$ interneurons are implicated in locomotion as indicated by increased c-fos immunoreactivity following fictive locomotion (Lanuza et al., 2004). However, *Evx1* knockout mice have grossly normal locomotion patterns despite a ∼70% reduction in the $V0_V$ interneurons and loss of appropriate contralateral intersegmental axonal projections in the remaining ∼30% of interneurons (Moran-Rivard et al., 2001). A subset of the $V0_V$ class has been reported to be excitatory in an unpublished observation (Zhang et al., 2008).

$V0_D$

Unlike the $V0_V$ subclass, the more dorsal *Dbx1*$^+$ progenitors of the glycinergic/GABAergic $V0_D$ class do not express *Evx1* (Pierani et al., 2001; Lanuza et al., 2004). And while both $V0_D$ and $V0_V$ classes have similar axon guidance and cell body position, the loss of the $V0_D$ class, in conjunction with $V0_V$ class, does appear to alter locomotor behavior. When *Dbx1* is knocked out, eliminating all V0 progenitors, a disruption of left–right coordination is observed at lumbar levels L2 and L5. These periods of left–right synchrony are intermittent and periods of normal left–right alternation are observed amidst episodes of synchrony (Lanuza et al., 2004). No disruption of flexor-extensor behavior, as indicated by alternating phasic activity of the L2 and L5 segments, was observed in a drug-induced isolated cord fictive locomotion assay (Lanuza et al., 2004). Recently, studies have showed that a cluster of $V0_D$ cells lateral to the central canal receive substantial input from primary afferents and preferentially project axons toward contralateral motoneurons via an oligosynaptic pathway, and are active during fictive locomotion. This suggests that this subset of V0 interneurons may be primarily responsible for coordination of left–right alternation during locomotion (Griener et al., 2015).

$V0_C$ and $V0_G$

The $V0_C$ and $V0_G$ subclass represent ∼5% of V0 progenitors and are identified by expression of *Pitx2* and occupy a medial position dorsal to the central canal (Zagoraiou et al., 2009). These cells were first observed in lumbar levels at E11.5–12.0 by Pitx2 immunoreactivity which, unlike many embryonic markers, could be detected until postnatal day 30 (Zagoraiou et al., 2009). Neurotransmitter markers can subdivide the *Pitx2*$^+$ cells into cholinergic (*vAChT*$^+$ and *ChAT*$^+$) and glutamatergic (*vGluT2*$^+$) types that are distinct (Zagoraiou et al., 2009). While these are found at cervical and lumbar levels, within the lumbar cord, these two types are distributed in a gradient such that a greater number of cholinergic interneurons are found at more rostral levels and a greater number of glutamatergic interneurons at more caudal levels (Zagoraiou et al., 2009). The cholinergic cells are distinct from *Pitx2*$^-$ cholinergic C_3 propriospinal interneurons (Zagoraiou et al., 2009). By genetic tracing, ∼80% of these neurons were determined to be from a *Dbx1*$^+$ progenitor domain at E12.5 and loss of *Dbx1* eliminated the Pitx2 immunoreactivity in the intermediate cord. Because $V0_C$ and $V0_G$ *Pitx2*$^+$ cells transiently express *Evx1*, they appear to be subsets of the $V0_V$

class. This relatively small ipsilaterally and bilaterally projecting class, however, is responsible for perhaps all c-boutons on motor neurons found in P8 to P25 mice (Zagoraiou et al., 2009; Stepien et al., 2010). These interneurons provide relatively weak innervation to *Sox14::eGFP*$^+$ V2a and calbindin$^+$ V1 Renshaw cells. These cells appear to be involved in local circuitry as corticospinal and sensory *vGluT1*$^+$ glutamatergic boutons were not found, whereas serotonergic and *GAD67*$^+$ GABAergic boutons were observed (Zagoraiou et al., 2009). While previous experiments did not find a gross locomotor behavioral defect with loss of the V0$_V$ subclass in *Evx1* mutant animals, *Pitx2* mutant animals were found to have defects in locomotion revealed by EMG recordings during swimming (Zagoraiou et al., 2009). This deficit was argued to represent an abnormal integration of sensory inputs. It may, alternatively or in addition, represent a deficit in C-terminal modulation of motor neuron excitability. A survey in E12.5 mice showed that several V0 subclasses can be defined by expression of *Pax2, Pax6, Evx1, Ptx2, Nurr1, HNF-6, BhlhB5*, and *Prdm8* (Francius et al., 2013).

V1 Interneuron Characteristics

As a population, this group appears to control burst durations and is comprised of cells physiologists defined as Ia inhibitory and Renshaw cells. Mice models without V1 and V2b showed significant difficulty with limb articulation in flexion and extension (Zhang et al., 2014). The pV1 progenitor domain gives rise to important inhibitory subclasses of neurons that were previously described electrophysiologically: the Ia inhibitory interneurons that mediate reciprocal inhibition and the Renshaw cells that mediate inhibitory feedback to integrate limb and muscle length information into spinal circuitry. Renshaw cells and Ia inhibitory interneurons are V1 derived, but differ in morphology, location, calcium-binding protein expression, synaptic connectivity, and function. These differences are already present in neonates and their differentiation starts in the early embryo (Benito-Gonzalez and Alvarez, 2012). In addition, 75% of V1 interneurons are non-Ia, non-Renshaw subclasses that await characterization (Sapir et al., 2004; Alvarez et al., 2005). Short-range ipsilaterally and rostrally projecting glycinergic/GABAergic V1 neurons are characterized by transient expression of homeodomain transcription factor *En1* (**Figure 4C**; Burrill et al., 1997; Matise and Joyner, 1997; Pierani et al., 1999, 2001; Saueressig et al., 1999). Studies in embryonic chick indicate that these neurons project for only 1–2 segments and have been shown to make inhibitory contacts onto motor neurons and other interneurons, although this may not be the case in the mature mouse (Wenner et al., 1998, 2000). Loss of *Pax6* or *En1*-dependent DTA ablation eliminates the recurrent inhibition by Renshaw cells on motor neurons (Sapir et al., 2004; Gosgnach et al., 2006). Elimination of V1 interneurons results in a marked slowing on the drug-induced fictive locomotion period that is seen in conventional knockouts, targeted ablation, and acute inhibition with allatostatin (Gosgnach et al., 2006; Goulding, 2009). The mechanism by which elimination of an inhibitory class would prolong the locomotor cycle remains unknown and may result from the loss of inhibitory neurons to terminate MN firing. The V1 class, like V0, expresses the inhibitory spinal interneuron markers *Lhx1* and *Lhx5* (Pillai et al., 2007).

V1 Birth and Early Development

Unlike cells in the dorsal-most p0 domain that expresses *Dbx1* and *Dbx2*, the adjacent p1 domain only expresses *Dbx2* (Pierani et al., 1999). The V1 class appears from a *Pax6*$^+$, *Dbx2*$^+$, *Nkx6.2*$^+$, *Dbx1*$^-$ domain that is ventral to the *Dbx1/2*$^+$ V0 domain (Matise and Joyner, 1997; Pierani et al., 1999). In chick, *En1*$^+$, *Lim1/2*$^+$ V1 neurons appear at stage 17, and most appear ventral to the domain of *Dbx1* expression, within the ventral domain of these *Dbx2*$^+$, *Dbx1*$^-$ progenitors (Pierani et al., 1999). *Dbx* expression does not overlap with *En1*, perhaps due to the relatively late expression of *En1* (Pierani et al., 1999). Genetic tracing studies using *Dbx1*^{nlsLacZ} mice between ages E10 and E16.5 found that ∼5–10% of *En1*$^+$ cells did express a low level of βgal, perhaps a reflection of transient *Dbx1* expression and more enduring βgal protein. The V1 class is marked by expression of *Foxd3*, found in the dI2 domain, as well (Ramos et al., 2010). The transcription factor, *Bhlhb5*, which marks the V1, V2 and dI6 domains, is required at least partially for V1 identity assessed by *En1* expression (Ramos et al., 2010; Skaggs et al., 2011). Expression of *Bhlhb5* in conjunction with *Ngn2* facilitates V1 identity ectopically (Skaggs et al., 2011).

V1 Interneuron Subtypes

V1 Renshaw

Renshaw cells use both glycine and GABA as neurotransmitters, transiently express *Gad65* early in embryonic development and have both motor neurons and Ia interneurons as targets (Saueressig et al., 1999; Sapir et al., 2004). They also express calbindin D28K embryonically and continue to express this marker into adulthood (Alvarez et al., 1999; Geiman et al., 2000). They receive input from motor neuron collaterals that release acetylcholine, glutamate, and aspartate (Mentis et al., 2005; Richards et al., 2014). Renshaw cells modulate proprioceptive sensory input and motor neuron output. Genetic tracing studies showed that Renshaw cells are derived from an *En1*$^+$ progenitor pool and, although they are not lost in the absence of *En1*, they do have fewer motor neuron recurrent inputs (Sapir et al., 2004). They are, however, lost in the absence of *Pax6* (Sapir et al., 2004). Recent study showed that selective activation of the Onecut transcription factors Oc1 and Oc2 during the first wave of V1 interneuron neurogenesis is a key step in the Renshaw cell differentiation; furthermore Renshaw cell development is dependent on the forkhead transcription factor Foxd3, which is more broadly expressed in post-mitotic V1 interneurons (Stam et al., 2012).

V1 Ia Interneuron

Although Ia interneurons have been rediscovered as a V1 subclass, like Renshaw cells, the Ia INs were functionally described before the advent of molecular genetic dissection of interneuron development (Eccles et al., 1954; Hultborn et al., 1971; Hultborn and Udo, 1972) These inhibitory glycinergic cells receive input from muscle spindle Ia proprioceptive afferents carrying muscle length information and provide inhibitory

input onto motor neurons innervating antagonist muscles. Like motor neurons, Ia receive inhibitory inputs from Renshaw cells (Hultborn et al., 1971). In neonatal mice, disynaptic glycinergic reciprocal inhibition is mediated by Ia interneurons, although this activity is preserved in the absence of *Pax6*, indicating that cells of more than one origin contributes to this functional class (Wang et al., 2008). Only when V1 and V2b are both ablated is reciprocal inhibition profoundly altered. Renshaw cells constitute 8–19% of V1 interneurons and the *Foxp2*$^+$ (by immunohistochemistry) population accounts for around 33% of these neurons at P0 and 50% at E13 (Morikawa et al., 2009). Because there are no universal markers of Ia interneurons, all Ia interneurons cannot be accounted for, leaving the physiologic properties and connectivity patterns of V1 interneurons unaccounted for (Alvarez et al., 2005). Of note, some interneurons with synaptic organization like Ia interneurons have been found that arise from the V1 population and are Foxp2 positive (Morikawa et al., 2009). A survey in E12.5 mice showed that several V1 subclasses can be defined by expression of *Calbindin, OC1, OC2, OC3, Foxd3, En1, MafB, FoxP2, Foxd3, Foxp4, Pax2, Arx, Evx1, Nurr1, BhlhB5, Pou4F1, Pou3F1,* and *Prdm8* (Francius et al., 2013).

V2 Interneuron Characteristics

V2 interneurons become divided into V2a and V2b classes of ipsilaterally projecting interneurons that extend axons caudally across several segments (Goulding, 2009). The excitatory V2a class is glutamatergic and expresses *Chx10*, while the *Gata2/3*$^+$ V2b class is inhibitory and uses both glycine and GABA (**Figure 4D**; Al-Mosawie et al., 2007; Lundfald et al., 2007). The transcription factor, *Bhlhb5*, marks the V2, as well as V1 and dI6 domains (Ramos et al., 2010).

V2 Birth and Early Development (Notch-Delta)

V2 interneurons arise from a progenitor pool just dorsal to the pMN domain and share expression of *Lhx3* with the pMN domain. In addition, both domains share expression of NLI that forms homodimers. This NLI homodimer nucleates the formation of a higher-order tetramer with Lhx3 in the V2 progenitor domain, and in the case of pMNs this V2-defining tetramer (Lhx3-NLI-NLI-Lhx3) is disrupted by the insertion of Isl1 to form a hexamer (Lhx3-Isl1-NLI-NLI-Isl1-Lhx3). Transcriptional response elements that are active in V2 cells can bind both the motor neuron hexamers and the V2 associated tetramers, while response elements active in motor neurons are only responsive to the hexamers (Lee et al., 2008). Later the V2 domain expresses *Chx10* that acts as a repressor of motor neuron associated hexamers in V2 progenitors, leaving only the LIM tetramers active (Sander et al., 2000; Lee et al., 2008). The progenitor pool of V2 neurons becomes post-mitotically segregated into V2a and V2b neurons.

Time-lapse imaging in zebrafish showed that the majority of V2 progenitors give rise to a pair of V2a and V2b cells (Kimura et al., 2008), indicating that V2a and V2b arise from the same progenitor. This segregation into V2a and V2b is mediated by Notch/delta signaling in zebrafish and mouse models (Yang et al., 2006; Del Barrio et al., 2007; Peng et al., 2007). In mouse, Delta4, but not Delta 1, activates this signaling cascade and is downstream of *Foxn4,* which also induces expression of *Mash1/Ascl1* (Del Barrio et al., 2007; Peng et al., 2007). Mind bomb-1 (Mib1) is an E3 ubiquitin ligase that ubiquitinates and promotes the endocytosis of Notch ligands. In mice model, Mib1 plays an important role in Notch activity and specific differentiation, neurogenesis and gliogenesis of V2 interneurons. Mice models with abnormal Mib1 resulted in unclear spinal progenitors, premature or unbalanced differentiation or loss of astrocytes and oligodendrocytes (Kang et al., 2013). In zebrafish embryos two ligands, DeltaA and DeltaD, and three receptors, Notch1a, Notch1b, and Notch3 redundantly contribute to p2 progenitor maintenance; on the other hand, DeltaA, DeltaC, and Notch1a mainly contribute to the V2a/V2b cell fate determination (Okigawa et al., 2014). Misra et al. (2009) showed Foxn4 and proneural factors may serve as the trigger to initiate asymmetric Dll4-Notch and subsequent BMP/TGFβ signaling events required for neuronal diversity in the V2 domain (Okigawa et al., 2014). V2b fate is specified by active *Notch1, Foxn4, Mash1,* and *Scl* Notch-binding protein MAML is also required for this specification (Peng et al., 2007). Lack of active Notch1 results in V2a fate, shown in an increase of V2a interneurons at the expense of V2b in *Psn1* KO mice or *Notch1* KO mice (Del Barrio et al., 2007; Peng et al., 2007). Transcription factor Gata2 is necessary in the normal development of V2a and V2b neurons and Gata2 promotes the selective activation of V2b at the expense of V2a fate (Francius et al., 2014). Progenitors that express the notch ligand, Delta-like 4 generate almost all V2a and V2c neurons while producing only a small fraction of neurons of other subtypes along the dorsoventral axis (Zou et al., 2015).

V2 Interneuron Subtypes

V2a Sox14/Chx10

The V2a class of ipsilaterally projecting interneurons expresses the transcription factors Chx10 and Sox14 and is glutamatergic. These interneurons are composed of cells with diverse firing properties and morphologies with local as well as long-range ipsilateral projection patterns (Dougherty and Kiehn, 2010a,b; Zhong et al., 2010). This class of interneurons has been shown to contact motor neurons (Al-Mosawie et al., 2007; Stepien et al., 2010) and contralaterally projecting V0 interneurons (Crone et al., 2008). Loss of these cells has been shown to disturb locomotor function in a state-dependent manner (Crone et al., 2008, 2009; Dougherty and Kiehn, 2010a,b; Zhong et al., 2010). In a series of *in vitro* experiments it was found that *Chx10-DTA* V2a-ablated mice displayed more variable amplitude and period than wild-type controls during drug-induced fictive locomotion. Further, these mutant animals had incoherent left–right alternation during drug-induced fictive locomotion. Surprisingly, these animals failed to display coordinated brainstem stimulated or dorsal root stimulated fictive locomotion, suggesting that *Chx10*$^+$ cells mediate descending and sensory activation of locomotor activity (Crone et al., 2008).

A subsequent study, using a different strain of mice that avoided the neonatal lethality seen in previous work, showed that during treadmill running, *Chx10-DTA* mice can transition from

alternating locomotion to synchronous hindlimb locomotion at higher speeds. High-speed synchronous left–right activity, or galloping, is not normally seen in mice, although it has been described in studies of *Eph* and *ephrin* signaling molecule mutant mice (Dottori et al., 1998; Kullander et al., 2001; Yokoyama et al., 2001). The *Eph/ephrin* mutant mice, however, have synchronous activity at both slow and fast speeds. Some V2a interneurons express *EphA4*, but a compelling correlation has yet to be discovered (Lundfald et al., 2007). In zebrafish, *alx,* a zebrafish homolog of *Chx10*, is expressed in an ipsilateral descending excitatory interneuron population named CiD (circumferential descending) neurons that monosynaptically contact motor neurons (Kimura et al., 2006; McLean et al., 2008). This population has been shown to be active during high-frequency swimming in larval zebrafish (McLean et al., 2008). Within this interneuron class, dorsally located cells are recruited at a high swimming frequency. As the frequency decreases, more ventral cells are recruited, accompanied by silencing of previously active dorsal cells (McLean et al., 2008). A survey in E12.5 mice showed that V2a subclasses can be defined by expression of *BhlhB5, Pou3F1, OC1, OC2, OC3, Prdm8, MafA,* and *cMaf* (Francius et al., 2013).

V2b Gata2/3

Ipsilaterally projecting V2b interneurons express *Gata2/3*, are inhibitory GABAergic neurons, and appear to make direct connections onto motor neurons (Lundfald et al., 2007; Peng et al., 2007). Observations by the Goulding lab indicate they project caudally (Zhang et al., 2014). These cells may underlie the retained reciprocal inhibitory pathways seen in V1 knockout mice (Wang et al., 2008). A survey in E12.5 mice showed that V2b subclasses can be defined by expression of *BhlhB5, Pou3F1, OC1, OC2, OC3, Prdm8, MafA,* and *MafB* (Francius et al., 2013). As pointed out earlier, V1- and V2b-derived neurons function as the core interneuronal components of the limb central pattern generator (CPG) that coordinate flexor-extensor motor activity (Zhang et al., 2014).

V2c Sox1

The V2 interneuron class has recently been shown to further diverge to a Sox1-expressing Gata3-negative population named V2c interneurons, function of which is still yet to be elucidated (Li et al., 2010; Panayi et al., 2010). A survey in E12.5 mice showed that V2c subclasses can be defined by expression of *Sox1, OC1, OC2, and OC3* (Francius et al., 2013).

V3 Interneuron Characteristics

The $Sim1^{+}$ $VGluT2^{+}$ glutamatergic V3 interneurons send projections predominantly contralaterally and caudally (Goulding, 2009). Genetic tracing, using a *Sim1-eGFP* or $Sim1^{Cre}$ and reporter lines, and viral tracing, using pseudorabies, shows that 80–85% of these cells project contralaterally and a minor proportion remain ipsilateral or project both contra- and ipsilaterally (Zhang et al., 2008). As a population, $Sim1^{+}$ V3 interneurons form 24% of glutamatergic connections on V1 Ia, 27% on Renshaw subclasses, 22% of glutamatergic synapses on lateral motor column motor neurons, as well as connections on $Lhx3^{+}$ V2 interneurons, and lamina VIII commissural interneurons (Zhang et al., 2008). Behaviorally, loss of V3 neuronal activity by genetic attenuation with tetanus toxin or allatostatin signaling resulted in a loss of CPG robustness. In isolated cord fictive locomotion, both dorsal root stimulation and drug-induced methods produced weak CPG activity in only some of the cords examined. The outputs were less consistent and had greater coefficients of variance. Although both right and left sides of the cord produced irregular outputs, the fidelity of left–right coordination was preserved suggesting that V3 interneurons do not regulate the coordination of left–right activity. In adult $Sim1^{Cre}$ *AlstR192* animals, application of allatostatin to the cord produced locomotor disturbances in gait, as well (Zhang et al., 2008). In Sim1 mutant mice, V3 interneurons are produced normally and maintain in the similar position and organizations as wild-type; however, there is significant reduction of interneurons in dorsal subgroup and there is significant reduction in the contralateral axonal projection. Therefore, Sim1 appears to be critical in migration and axonal projection of V3 interneuronal development (Blacklaws et al., 2015). Mice that are mutant for *Nkx2.2* and *Nkx2.9* lose V3 interneurons and $Nkx2.2^{+/-}$ $Nkx^{-/-}$ mice display intermittent or permanent hopping gait (Holz et al., 2010). Holz et al. (2010) indicate that this mutation affects floor plate, and therefore likely affects commissural interneuron projections that mediate left–right coordination. A survey in E12.5 mice showed that V2c subclasses can be defined by expression of *Olig3, Prox1, BhlhB5,* and *Nurr1* (Francius et al., 2013).

V3 Birth and Early Development

These V3 interneurons arise from the ventral-most p3 progenitor domain defined by homeobox transcription factors *Nkx2.2* and *Nkx2.9* and the PAS-bHLH transcription factor *Sim1* (simple-minded homolog 1; **Figure 4E**; Briscoe et al., 1999; Goulding et al., 2002). Genetic tracing techniques using a $Sim1^{TauLacZ}$ knock-in reporter mouse or $Sim1^{Cre}$ and reporter lines ($R26^{floxstop-GAP43-GFP}$ and $R26^{floxstop-lacZ}$) have shown similar expression at E11.5 to *in situ* hybridization data for *Sim1* expression that appeared just lateral to the *Nkx2.2* progenitors (Marion et al., 2005; Zhang et al., 2008). *Nkx2.2* also regulates the expression of *Olig3* in V3 neurons. While *Olig3* plays a key role in respecification of dl2 and dl3 neurons into dl4 interneurons in dorsal spinal cord (see below), it does not appear to affect the generation and migration of the ventral neurons (Liu et al., 2014).

$V3_D$ and $V3_V$

Each class of interneurons can likely be further subdivided. The existence of V3 subtype heterogeneity defined by cell body positions was first reported in a review of locomotor circuitry by the Goulding group (Goulding, 2009). This group recently examined both electrophysiological and morphological properties of mature V3 interneurons in adult mouse and were able to identify two V3 subpopulations with distinct intrinsic properties and distributions (ventral and dorsal), as well as an important intermediate subgroup (Borowska et al., 2013). They

reported $V3_V$, primarily located in lamina VIII, possessed a few branching processes and were capable of generating rapid tonic firing spikes and $V3_D$ had a more complex morphology with relatively slow average spike frequency with strong adaptation (Borowska et al., 2013). A survey in E12.5 mice showed that $V3_V$ express *Olig3, Prox1, BhlhB5,* and *Nurr1*, and $V3_D$ can be defined by expression of *OC1, OC2,* and *OC3* (Francius et al., 2013).

V_X Hb9

A group of glutamatergic, rhythmically active interneurons with possible connections to motor neurons can be found along either side of the ventral midline in thoracic and upper lumbar segments (Thaler et al., 1999; Wichterle et al., 2002; Hinckley et al., 2005; Wilson et al., 2005). These $Hb9^+$ and $VGluT2^+$ interneurons are found in lamina VIII, although the developmental origin of these cells is unknown (**Figure 4F**). These cells have oscillatory behavior, make potential contacts with motor neurons, and are associated with motor rhythms (Hinckley et al., 2005; Wilson et al., 2005; Hinckley and Ziskind-Conhaim, 2006). These interneurons were the first to show oscillatory properties and efforts have been made to discover a relationship to rhythm generation or a pacemaker property for the CPG (Kwan et al., 2009). No cell class, however, has been found to act as a pacemaker for CPG activity. Remaining questions for the V_X include: what is the progenitor domain that gives rise to the V_X domain; and why are they not found below the L2 segment at E18.5.

Dorsal Interneuron Progenitors

There are eight canonical classes of dorsal progenitors, dI1–6 and dIL_A and dIL_B. Of these, the dorsal-most dI1–3 progenitors are dependent on signals from the roof plate and termed Class A (Liem et al., 1997; Lee et al., 2000). The remaining dI4–6 and dIL_A and dIL_B are independent of roof plate signals and termed Class B (Gross et al., 2002; Muller et al., 2002). The dorsal-most progenitors, pd1–pd3, are born between days E9.5 and 10.5 and become post-mitotic and begin to migrate ventrally between E10.5 and E11.5 (Helms and Johnson, 1998; Bermingham et al., 2001; Gross et al., 2002; Muller et al., 2002). These cells will eventually form the deeper layers of the dorsal horn. The more ventral Class B dI4–6 cells are born between E10 and 12.5 and then post-mitotically express *Lbx1* and migrate either dorsally to form the more superficial layers of the dorsal horn or migrate ventrally to the deep dorsal horn and the ventral spinal cord (Gross et al., 2002; Muller et al., 2002). The later born dIL_A and dIL_B classes are born between E11 and E13 and are intermixed with each other. They then migrate dorsally and constitute a significant portion of the cells in the superficial dorsal horn, including the substantia gelatinosa (Nornes and Carry, 1978; Gross et al., 2002; Muller et al., 2002; Mizuguchi et al., 2006). As with the ventral interneuron classes, each of these classes, or their subgroups, has characteristic features. For instance, each interneuron subclass appears to have a unique axonal projection that produces a tight fascicle within white matter tracts (Avraham et al., 2009, 2010).

dI1 Interneuron Characteristics

The dorsal-most progenitor domain pd1 expresses the bHLH transcription factor $Math1^+$ (Mouse atonal homolog 1, also known as *Atoh1*) and gives rise to at least two $VGluT2^+$ glutamatergic subclasses: dI1A and dI1B, characterized by Lim-HD expression and their spinocerebellar tract (SCT) contributions (**Figure 4G**). Recent study shows that *Msx1* and *Msx2*, two homeodomain transcription factors that are induced earlier than bHLH transcription factors, likely play a role as transcriptional activators of *Math1*/*Atoh1* in spinal cord development (Duval et al., 2014). The dI1A (also known as $dI1_{comm}$) neurons express the Lim-HD transcription factors $Lhx2_{high}$ and $Lhx9_{low}$, while dI1B (also known as $dI1_{ipsi}$) express the Lim-HD TF *Lhx9* (Helms and Johnson, 1998; Lee et al., 1998; Bermingham et al., 2001; Gowan et al., 2001; Wilson et al., 2008; Avraham et al., 2009). The dI1 interneurons migrate to the deep dorsal horn and intermediate gray where they receive proprioceptive input from the periphery and form commissural projections of dorsal and ventral SCTs (Helms and Johnson, 1998; Bermingham et al., 2001). Using an $Atoh1^{LacZ}$ allele to trace the fate of pd1 progenitors in developing mouse, at least two subsets of the dI1 class have been identified: (1) a medial cluster of vertically oriented neurons that are $Cbln2^+$ and $Smarca2^+$ and projects to the SCT in the contralateral ($Tag1^+$) lateral funiculus; (2) a more lateral $Sox6^+$, cluster of horizontally oriented neurons that contributes to the SCT in the ipsilateral lateral funiculus (Miesegaes et al., 2009). In chick, data with an enhancer that labels these cells suggests that both fascicles coalesce in the lateral funiculus ventral to the fascicle formed by the dI2 projections (Avraham et al., 2009).

dI1 Birth and Early Development

The roof-plate-dependent Class A dp1 progenitors of the dI1 class express the bHLH transcription factors *Olig3* and *Math1* (Muller et al., 2005; Gowan et al., 2001). The dI1 neurons in mouse are born between E10 and E12.5 and express *Lhx2/9*, *Barhl1* (bar homeobox like 1) and *Brn3a* (*Pou4f1*, a class IV POU domain-containing transcription factor; Helms and Johnson, 1998). Loss of function experiments with BMP7 in chick and *Bmp7* mutant mice results in loss of dI1, dI3, and dI5 (Le Dréau et al., 2012).

dI2 Interneuron Characteristics

dI2 interneurons are ascending, contralaterally projecting, relay interneurons that migrate to the intermediate spinal cord and ventral horn (Gowan et al., 2001; Gross et al., 2002). These interneurons have been suggested to convey sensory information via the spinothalamic tract to the thalamus, based on their location (**Figure 4E**; Brown, 1981; Tracey, 1985; Gross et al., 2002). The projections likely occupy the lateral funiculus and are dorsal to the dI1 fascicle, as analyzed by enhancer expression in chick (Avraham et al., 2009). Arising from bHLH transcription factor *Ngn1* (neurogenin 1) and *Ngn2* expressing progenitors, these neurons express LIM-HD transcription factors *Lhx1, Lhx5* and winged-helix domain *Foxd3* (forkhead homeobox D3) post-mitotically (Bermingham et al., 2001; Gowan et al., 2001; Gross et al., 2002). These interneurons were previously known as D3A interneurons.

dI2 Birth and Early Development

The roof-plate-dependent Class A dI2 progenitor domain, pd2, is characterized by the expression of the bHLH transcription factors, *Olig3*, *Ngn1*, and *Ngn2* and are born between E10- and E12.5 (**Figure 4H**; Gowan et al., 2001; Muller et al., 2005). Two SoxD transcription factors, Sox5 and Sox6, are expressed in restricted domains of dorsal progenitors. Sox5 controls cell fate specification of dp2 and dp3 progenitors and, as a result, controls the correct number of the corresponding dorsal interneurons (dI2 and dI3; Quiroga et al., 2015).

dI3 Interneuron Characteristics

The dI3 neurons are excitatory interneurons in the deep dorsal horn and intermediate spinal cord (Liem et al., 1997; Gowan et al., 2001; Cheng et al., 2004). These cells target motor neurons monosynaptically, as revealed by recent rabies tracing experiments (Stepien et al., 2010). They have axons that project rostrally, ipsilaterally, and longitudinally in two fascicles. A ventral fascicle enters the ventral lateral funiculus (VLF) and the dorsal fascicle enters the dorsal funiculus (DF; Avraham et al., 2010). The dorsal projecting axons re-enter the cord when they encounter axons sensory axons at the dorsal root entry zone (DREZ; Avraham et al., 2010). Similarly, the ventrally projecting neurons re-enter the cord at ventral root exit points (Avraham et al., 2010). In mice model, dl3 appears to convey input from low threshold cutaneous afferents to the motor neurons that is critical in hand/forelimb grip (Bui et al., 2013). The dI3 pool also expresses *Tlx3* (T-cell leukemia homeobox 3) and LIM-HD transcription factor *Isl1*-expressing cells (**Figure 4I**; Gross et al., 2002). The turning behavior of dI3 neurons is dependent on *Isl1*, and expression of *Isl1* in dI1 neurons conferred dI3-like axon choice points to dI1 neurons (Avraham et al., 2010). *Tlx1* (also known as *Hox11*) and *Tlx3* (also known as *Rnx* and *Hox11L2*) are markers of glutamatergic signaling. *Tlx3* functions cell-autonomously to specify a glutamatergic neurotransmitter phenotype (Cheng et al., 2004).

dI3 Birth and Early Development

The roof-plate dependent Class A dP3 progenitors express the basic helix-loop-helix (bHLH) transcription factor *Mash1* (*Ascl1*, Mouse Achaete-scute complex-like 1), as do adjacent pd4 and pd5 domains (Gowan et al., 2001; Helms et al., 2005). They also express *Olig3*, *Pax7* and *Ngn2* and *Gsh2* (Muller et al., 2005). In chick spinal cord electroporation experiments it has been shown that over-expression of *Olig3* increases dI3 interneurons at the expense of other Classes A and B neuron classes and this effect is enhanced by *Mash1* (Muller et al., 2005). Over-expression of *Mash1* results in more dI3 and dI5 neurons at the expense of dI2 and dI4 (Muller et al., 2005), while loss of Mash1 causes a decrease in dI3 and dI5 populations while dI4 is maintained (Helms et al., 2005). As mentioned above, loss of function experiments with BMP7 in chick and *Bmp7* mutant mice results in loss of dI1, dI3, and dI5 (Le Dréau et al., 2012).

dI4 Interneuron Characteristics

The early born (E10.5–E11) dI4 interneurons become $Pax2^+$, $Lhx1^+$, and $Lhx5^+$ GABAergic ipsilaterally projecting somatosensory associative neurons that migrate laterally to the deep dorsal horn (**Figure 4J**; Gross et al., 2002; Muller et al., 2002; Pillai et al., 2007). In addition, both dI4 and dI5 interneurons also express *Gsh1* (*Gsx1*) and *Gsh2* post-mitotically, while dI3 only express *Gsh2* (Kriks et al., 2005; Muller et al., 2005; Mizuguchi et al., 2006). They are GABAergic, calbindin$^+$ and express the nociceptive marker *PLCγ* (Chen et al., 2001; Helms and Johnson, 2003). The dI4 fate is dependent on *Ptf1a* and loss of this gene results in loss of all GABAergic dorsal neurons and respecification to dI5 fate (Henke et al., 2009; Meredith et al., 2009). Loss of *Lhx1* and *Lhx5* results in a loss of *Pax2*, *Viaat,* and *Gad1* (Pillai et al., 2007). In addition, *Pax2* is required for the maintained expression of *Lhx1, Lhx5, Pax5,* and *Pax8* (Pillai et al., 2007).

dI4 Birth and Early Development

The roof-plate independent Class B dP4 domain expresses *Lbx1*, *Mash1* and higher levels of *Pax7* than the dorsally adjacent pD3 domain (Gross et al., 2002; Muller et al., 2002). These progenitors are born between E10.5 and E11 and this domain is distinct from the dIL progenitor domain that produces dIL_A and dIL_B progenitors, although both have very similar transcription factor expression patterns (below). *Olig3* over-expression can inhibit formation of dI4, and loss can result in expansion of this domain (Muller et al., 2005). While these cells express *Mash1*, loss of *Mash1* does not block dI4 formation, yet it does disrupt dI3 and dI5 (Helms et al., 2005).

dI5 Interneuron Characteristics

The roof-plate independent Class B dI5 neurons become contralaterally projecting glutamatergic somatosensory interneurons of the deep dorsal horn (nucleus proprius) and ventral horn that express the homeodomain transcription factors *Lbx1*, *Brn3a*, *Tlx1*, *Tlx3,* and *Lmx1b* (**Figure 4K**; Gross et al., 2002; Muller et al., 2002; Qian et al., 2002; Ding et al., 2005; Glasgow et al., 2005). In addition, a subset expresses *PhoxA2* (Ding et al., 2004). These interneurons were previously known as D4.

dI5 Birth and Early Development

These cells are born between E10.5–E11 and arise from a $Mash1^+$ and $Pax7^+$ dP5 progenitor domain that express *Lbx1* post-mitotically to both reinforce Class B fate and oppose Class A fates (**Figure 4K**; Gross et al., 2002; Muller et al., 2002). The dI5 domain expresses *Gsh1* and *Gsh2*, as does the adjacent dI4 domain (Kriks et al., 2005). As noted previously, loss of function experiments with BMP7 in chick and *Bmp7* mutant mice results in loss of dI1, dI3, and dI5 (Le Dréau et al., 2012).

dI6 Interneuron Characteristics

The roof-plate independent Class B dI6 commissural inhibitory interneurons express *Lbx1*, *Lhx1*, *Lhx5*, and are *Pax2* positive, indicating a GABAergic fate (**Figure 4L**; Gross et al., 2002; Muller et al., 2002; Cheng et al., 2004; Glasgow et al., 2005; Pillai et al., 2007). These cells may also use glycine for neurotransmission (Goulding, 2009). Although arising from a dorsal progenitor pool, and not being part of the "V" interneurons, the dI6 group

of interneurons gives rise to more than one subtype and appears to contribute to motor function (Gross et al., 2002; Muller et al., 2002; Lanuza et al., 2004). These inhibitory neurons are reported in unpublished observations to be commissural and may be involved in right–left alternation, as well (Goulding, 2009). Dmrt3, a novel marker in dl6 interneuron was traced to play a key role in locomotor circuitry and in development of commissural interneurons, and mutation in dmrt3 result in divergent in gait pattern in mice models (Andersson et al., 2012; Vallstedt and Kullander, 2013). Double knockout of *Lhx1* and *Lhx5* results in a loss of *Pax2*, *Viaat,* and *Gad1* expression (Pillai et al., 2007). Furthermore, *Pax2* is required for the maintained expression of *Lhx1, Lhx5, Pax5,* and *Pax8* (Pillai et al., 2007). These cells also express *WT1* (Wilms' tumor 1; Goulding, 2009). The transcription factor, *Bhlhb5*, marks the dI6, V1 and V2 domains (Ramos et al., 2010). Electrophysiologic characteristics of the dl6 interneurons around a central canal reveal two possible subtypes: one firing trains of action potentials that are loosely coupled to the ventral root output and expressing intrinsic rhythmic activity which suggests a role in locomotor rhythm generation. The other subtype fires action potentials that are tightly coupled to the ventral root output (Dyck et al., 2012).

dI6 Birth and Early Development

The dI6 neurons are born around E10.5–E11 and originate from a $Pax7^+$, $Dbx2^+$, $Ngn1^+$ and $Ngn2^+$ pD6 progenitor domain. Post-mitotically they express *Bhlhb5*, *Wt1*, *Lbx1*, *Lhx1*, *Lhx5,* and *Pax2* (references above).

Late Born Dorsal Interneurons

The dIL neurons represent a second wave of neurogenesis from the dIL progenitor domain that constitutes most of the interneurons in the superficial dorsal horn in Rexed laminae II–IV (Gross et al., 2002; Muller et al., 2002). These cells are formed from common progenitors, and their cell fates are controlled by *Ascl1/Mash1* (**Figure 4M**; Mizuguchi et al., 2006).

dIL_A Interneuron Characteristics

The roof-plate independent Class B IL_A interneurons are ipsilaterally projecting association neurons that occupy the superficial dorsal horn in Rexed laminae I–III (Gross et al., 2000). These inhibitory neurons are GABAergic and are calbindin+, and a subset express *Gbx1* (John et al., 2005; Mizuguchi et al., 2006).

dIL_A Birth and Early Development

The dIL_A interneurons are late born (E.13.5) and arise from the dIL progenitor domain that is $Lbx1^+$, $Lhx1/5^+$, and $Pax2^+$(Pillai et al., 2007). The sensory relay interneuron maker *Foxd3* is downregulated in these cells (Gross et al., 2002). *Pax2* has been shown to be necessary for GABAergic differentiation and 98% of these cells also express *Gad1* (*GAD67*; Cheng et al., 2004). The dIL_A subclass is dependent on *Ptf1a* and loss of this gene results in trans-fate to the dIL^B identity (Glasgow et al., 2005).

dIL_B Interneuron Characteristics

The roof-plate independent Class B are $DRG11^+$ ipsilaterally projecting association interneurons that integrate input from cutaneous sensory neurons that detect noxious stimuli (Chen et al., 2001; Gross et al., 2002). This last-born subclass gives rise to glutamatergic neurons expressing *Tlx1* and *Tlx3* and *Lmx1b* (Cheng et al., 2004; Mizuguchi et al., 2006). The dIL_B neurons migrate dorsolaterally to settle in the superficial dorsal horn in Rexed laminae I–III (Gross et al., 2000; Muller et al., 2002). Over 96% of the dorsolateral population of these cells expresses both *Tlx3* and *VGluT2* (Cheng et al., 2004; Glasgow et al., 2005).

dIL_B Birth and Early Development

These neurons are born later than the dI1–6 class and express *Lbx1* post-mitotically (Gross et al., 2000; Muller et al., 2002). Mash1 controls the upregulation of Notch signaling to direct formation of dILB from common dIL progenitors (Mizuguchi et al., 2006).

Discussion

Combinatorial transcriptional control of cell fate is a mature perspective for understanding spinal cord development. This focus on transcription factors has been powerful in two major respects. First, it has allowed the identification of downstream factors that direct cell-specific characteristics, such as neurotransmitter status (Cheng et al., 2004). Second, it has permitted a powerful genetic analysis of spinal neurons, using transcription factors as class-specific tools to drive changes in cell fate and function (Lee et al., 2000; Zhang et al., 2008). Novel techniques are emerging for tracing neuronal circuitry and for the sophisticated manipulation of neuronal activity, including selective cellular ablation, optogenetic activation and silencing, and chemically induced activation and silencing (Boyden et al., 2005; Wickersham et al., 2007; Alexander et al., 2009). These techniques may reach their most exciting potential when coupled with the increasingly specific genetic control available in the spinal cord.

The further refinement of developmental neuronal classes is showing that subclasses may reflect functionally coherent groups of cells that can be mapped onto physiologically identified populations (**Figure 5**). Therefore, as the spinal cord development field grows to incorporate circuitry and behavior, it is merging with the rich field of adult spinal cord electrophysiology that has uncovered major mechanisms of spinal cord function. The combination of these disciplines will advance spinal cord biology to a state that fully encompasses both form and function.

Acknowledgments

We apologize to the researchers whose work was not included due to space constraints. We thank Samuel Pfaff for support; and Ariel Levine, Marito Hayashi, and members of the Pfaff Laboratory for critical reading of this manuscript.

References

Al-Mosawie, A., Wilson, J. M., and Brownstone, R. M. (2007). Heterogeneity of V2-derived interneurons in the adult mouse spinal cord. *Eur. J. Neurosci.* 26, 3003–3015. doi: 10.1111/j.1460-9568.2007.05907.x

Alaynick, W. A., Jessell, T. M., and Pfaff, S. L. (2011). SnapShot: spinal cord development. *Cell* 146, 178–178. doi: 10.1016/j.cell.2011.06.038

Alexander, G. M., Rogan, S. C., Abbas, A. I., Armbruster, B. N., Pei, Y., Allen, A. J., et al. (2009). Remote control of neuronal activity in transgenic mice expressing evolved G protein-coupled receptors. *Neuron* 63, 27–39. doi: 10.1016/j.neuron.2009.06.014

Alvarez, F. J., Dewey, D. E., McMillin, P., and Fyffe, R. E. (1999). Distribution of cholinergic contacts on Renshaw cells in the rat spinal cord: a light microscopic study. *J. Physiol.* 515(Pt 3), 787–797. doi: 10.1111/j.1469-7793.1999.787ab.x

Alvarez, F. J., Jonas, P. C., Sapir, T., Hartley, R., Berrocal, M. C., Geiman, E. J., et al. (2005). Postnatal phenotype and localization of spinal cord V1 derived interneurons. *J. Comp. Neurol.* 493, 177–92. doi: 10.1002/cne.20711

Andersson, L. S., Larhammar, M., Memic, F., Wootz, H., Schwochow, D., Rubin, C.-J., et al. (2012). Mutations in DMRT3 affect locomotion in horses and spinal circuit function in mice. *Nature* 488, 642–646. doi: 10.1038/nature 11399

Arber, S., Han, B., Mendelsohn, M., Smith, M., Jessell, T. M., and Sockanathan, S. (1999). Requirement for the homeobox gene Hb9 in the consolidation of motor neuron identity. *Neuron* 23, 659–674. doi: 10.1016/S0896-6273(01)80026-X

Ashrafi, S., Lalancette-Hebert, M., Friese, A., Sigrist, M., Arber, S., Shneider, N. A., et al. (2012). Wnt7A identifies embryonic gamma-motor neurons and reveals early postnatal dependence of gamma-motor neurons on a muscle spindle-derived signal. *J. Neurosci.* 32, 8725–8731. doi: 10.1523/JNEUROSCI.1160-12.2012

Avraham, O., Hadas, Y., Vald, L., Hong, S., Song, M. R., and Klar, A. (2010). Motor and dorsal root ganglion axons serve as choice points for the ipsilateral turning of dI3 axons. *J. Neurosci.* 30, 15546–15557. doi: 10.1523/JNEUROSCI.2380-10.2010

Avraham, O., Hadas, Y., Vald, L., Zisman, S., Schejter, A., Visel, A., et al. (2009). Transcriptional control of axonal guidance and sorting in dorsal interneurons by the Lim-HD proteins Lhx9 and Lhx1. *Neural Dev.* 4:21. doi: 10.1186/1749-8104-4-21

Benito-Gonzalez, A., and Alvarez, F. J. (2012). Renshaw cells and Ia inhibitory interneurons are generated at different times from p1 progenitors and differentiate shortly after exiting the cell cycle. *J. Neurosci.* 32, 1156–1170. doi: 10.1523/JNEUROSCI.3630-12.2012

Bermingham, N. A., Hassan, B. A., Wang, V. Y., Fernandez, M., Banfi, S., Bellen, H. J., et al. (2001). Proprioceptor pathway development is dependent on Math1. *Neuron* 30, 411–422. doi: 10.1016/S0896-6273(01)00305-1

Blacklaws, J., Deska-Gauthier, D., Jones, C. T., Petracca, Y. L., Liu, M., Zhang, H., et al. (2015). Sim1 is required for the migration and axonal projections of V3 interneurons in the developing mouse spinal cord. *Dev. Neurobiol.* doi: 10.1002/dneu.22266 [Epub ahead of print].

Borowska, J., Jones, C. T., Zhang, H., Blacklaws, J., Goulding, M., and Zhang, Y. (2013). Functional subpopulations of V3 interneurons in the mature mouse spinal cord. *J. Neurosci.* 33, 18553–18565. doi: 10.1523/JNEUROSCI.2005-13.2013

Boyden, E. S., Zhang, F., Bamberg, E., Nagel, G., and Deisseroth, K. (2005). Millisecond-timescale, genetically targeted optical control of neural activity. *Nat. Neurosci.* 8, 1263–1268. doi: 10.1038/nn1525

Briscoe, J., Pierani, A., Jessell, T. M., and Ericson, J. (2000). A homeodomain protein code specifies progenitor cell identity and neuronal fate in the ventral neural tube. *Cell* 101, 435–45. doi: 10.1016/S0092-8674(00)80853-3

Briscoe, J., Sussel, L., Serup, P., Hartigan-O'Connor, D., Jessell, T. M., Rubenstein, J. L., et al. (1999). Homeobox gene Nkx2.2 and specification of neuronal identity by graded Sonic hedgehog signalling. *Nature* 398, 622–627. doi: 10.1038/19315

Brown, A. G. (1981). *Organization of the Spinal Cord: The Anatomy and Physiology of Identified Neurones*. New York, NY: Springer-Verlag, 238. doi: 10.1007/978-1-4471-1305-8

Bui, T. V., Akay, T., Loubani, O., Hnasko, T. S., Jessell, T. M., and Brownstone, R. M. (2013). Circuits for grasping: spinal dI3 interneurons mediate cutaneous control of motor behavior. *Neuron* 78, 191–204. doi: 10.1016/j.neuron.2013.02.007

Burrill, J. D., Moran, L., Goulding, M. D., and Saueressig, H. (1997). PAX2 is expressed in multiple spinal cord interneurons, including a population of EN1+ interneurons that require PAX6 for their development. *Development* 124, 4493–4503.

Chen, Z. F., Rebelo, S., White, F., Malmberg, A. B., Baba, H., Lima, D., et al. (2001). The paired homeodomain protein DRG11 is required for the projection of cutaneous sensory afferent fibers to the dorsal spinal cord. *Neuron* 31, 59–73. doi: 10.1016/S0896-6273(01)00341-5

Cheng, L., Arata, A., Mizuguchi, R., Qian, Y., Karunaratne, A., Gray, P. A., et al. (2004). Tlx3 and Tlx1 are post-mitotic selector genes determining glutamatergic over GABAergic cell fates. *Nat. Neurosci.* 7, 510–517. doi: 10.1038/nn1221

Crone, S. A., Quinlan, K. A., Zagoraiou, L., Droho, S., Restrepo, C. E., Lundfald, L., et al. (2008). Genetic ablation of V2a ipsilateral interneurons disrupts left-right locomotor coordination in mammalian spinal cord. *Neuron* 60, 70–83. doi: 10.1016/j.neuron.2008.08.009

Crone, S. A., Zhong, G., Harris-Warrick, R., and Sharma, K. (2009). In mice lacking V2a interneurons, gait depends on speed of locomotion. *J. Neurosci.* 29, 7098–7109. doi: 10.1523/JNEUROSCI.1206-09.2009

Dasen, J. S., De Camilli, A., Wang, B., Tucker, P. W., and Jessell, T. M. (2008). Hox repertoires for motor neuron diversity and connectivity gated by a single accessory factor, FoxP1. *Cell* 134, 304–316. doi: 10.1016/j.cell.2008.06.019

Dasen, J. S., and Jessell, T. M. (2009). Hox networks and the origins of motor neuron diversity. *Curr. Top. Dev. Biol.* 88, 169–200. doi: 10.1016/S0070-2153(09)88006-X

Dasen, J. S., Tice, B. C., Brenner-Morton, S., and Jessell, T. M. (2005). A Hox regulatory network establishes motor neuron pool identity and target-muscle connectivity. *Cell* 123, 477–491. doi: 10.1016/j.cell.2005.09.009

Del Barrio, M. G., Taveira-Marques, R., Muroyama, Y., Yuk, D. I., Li, S., Wines-Samuelson, M., et al. (2007). A regulatory network involving Foxn4, Mash1 and delta-like 4/Notch1 generates V2a and V2b spinal interneurons from a common progenitor pool. *Development* 134, 3427–3436. doi: 10.1242/dev.005868

De Marco Garcia, N. V., and Jessell, T. M. (2008). Early motor neuron pool identity and muscle nerve trajectory defined by postmitotic restrictions in Nkx6.1 activity. *Neuron* 57, 217–231. doi: 10.1016/j.neuron.2007.11.033

Ding, Y. Q., Kim, J. Y., Xu, Y. S., Rao, Y., and Chen, Z. F. (2005). Ventral migration of early-born neurons requires Dcc and is essential for the projections of primary afferents in the spinal cord. *Development* 132, 2047–2056. doi: 10.1242/dev.01798

Ding, Y. Q., Yin, J., Kania, A., Zhao, Z. Q., Johnson, R. L., and Chen, Z. F. (2004). Lmx1b controls the differentiation and migration of the superficial dorsal horn neurons of the spinal cord. *Development* 131, 3693–3703. doi: 10.1242/dev.01250

Dottori, M., Hartley, L., Galea, M., Paxinos, G., Polizzotto, M., Kilpatrick, T., et al. (1998). EphA4 (Sek1) receptor tyrosine kinase is required for the development of the corticospinal tract. *Proc. Natl. Acad. Sci. U.S.A.* 95, 13248–13253. doi: 10.1073/pnas.95.22.13248

Dougherty, K. J., and Kiehn, O. (2010a). Firing and cellular properties of V2a interneurons in the rodent spinal cord. *J. Neurosci.* 30, 24–37. doi: 10.1523/JNEUROSCI.4821-09.2010

Dougherty, K. J., and Kiehn, O. (2010b). Functional organization of V2a-related locomotor circuits in the rodent spinal cord. *Ann. N. Y. Acad. Sci.* 1198, 85–93. doi: 10.1111/j.1749-6632.2010.05502.x

Duval, N., Daubas, P., Bourcier de Carbon, C., St Cloment, C., Tinevez, J. Y., Lopes, M., et al. (2014). Msx1 and Msx2 act as essential activators of Atoh1 expression in the murine spinal cord. *Development* 141, 1726–1736. doi: 10.1242/dev.099002

Dyck, J., Lanuza, G. M., and Gosgnach, S. (2012). Functional characterization of dI6 interneurons in the neonatal mouse spinal cord. *J. Neurophysiol.* 107, 3256–3266. doi: 10.1152/jn.01132.2011

Eccles, J. C., Fatt, P., and Koketsu, K. (1954). Cholinergic and inhibitory synapses in a pathway from motor-axon collaterals to motoneurones. *J. Physiol.* 126, 524–562. doi: 10.1113/jphysiol.1954.sp005226

Ericson, J., Morton, S., Kawakami, A., Roelink, H., and Jessell, T. M. (1996). Two critical periods of Sonic Hedgehog signaling required for the specification of motor neuron identity. *Cell* 87, 661–673. doi: 10.1016/S0092-8674(00)81386-0

Ericson, J., Thor, S., Edlund, T., Jessell, T. M., and Yamada, T. (1992). Early stages of motor neuron differentiation revealed by expression of homeobox gene Islet-1. *Science* 256, 1555–1560. doi: 10.1126/science.1350865

Francius, C., and Clotman, F. (2010). Dynamic expression of the Onecut transcription factors HNF-6, OC-2 and OC-3 during spinal motor neuron development. *Neuroscience* 165, 116–129. doi: 10.1016/j.neuroscience.2009.09.076

Francius, C., Harris, A., Rucchin, V., Hendricks, T. J., Stam, F. J., Barber, M., et al. (2013). Identification of multiple subsets of ventral interneurons and differential distribution along the rostrocaudal axis of the developing spinal cord. *PLoS ONE* 8:e70325. doi: 10.1371/journal.pone.0070325

Francius, C., Ravassard, P., Hidalgo-Figueroa, M., Mallet, J., Clotman, F., and Nardelli, J. (2014). Genetic dissection of Gata2 selective functions during specification of V2 interneurons in the developing spinal cord. *Dev. Neurobiol*. doi: 10.1002/dneu.22244 [Epub ahead of print].

Friese, A., Kaltschmidt, J. A., Ladle, D. R., Sigrist, M., Jessell, T. M., and Arber, S. (2009). Gamma and alpha motor neurons distinguished by expression of transcription factor Err3. *Proc .Natl. Acad. Sci. U.S.A.* 106, 13588–13593. doi: 10.1073/pnas.0906809106

Geiman, E. J., Knox, M. C., and Alvarez, F. J. (2000). Postnatal maturation of gephyrin/glycine receptor clusters on developing Renshaw cells. *J. Comp. Neurol.* 426, 130–142. doi: 10.1002/1096-9861(20001009)426:1<130::AID-CNE9>3.0.CO;2-7

Glasgow, S. M., Henke, R. M., Macdonald, R. J., Wright, C. V., and Johnson, J. E. (2005). Ptf1a determines GABAergic over glutamatergic neuronal cell fate in the spinal cord dorsal horn. *Development* 132, 5461–5469. doi: 10.1242/dev.02167

Gosgnach, S., Lanuza, G. M., Butt, S. J., Saueressig, H., Zhang, Y., Velasquez, T., et al. (2006). V1 spinal neurons regulate the speed of vertebrate locomotor outputs. *Nature* 440, 215–219. doi: 10.1038/nature04545

Gould, T. W., Buss, R. R., Vinsant, S., Prevette, D., Sun, W., Knudsonet, M., et al. (2006). Complete dissociation of motor neuron death from motor dysfunction by Bax deletion in a mouse model of ALS. *J. Neurosci.* 26, 8774–8786. doi: 10.1523/JNEUROSCI.2315-06.2006

Gould, T. W., Yonemura, S., Oppenheim, R. W., Ohmori, S., and Enomoto, H. (2008). The neurotrophic effects of glial cell line-derived neurotrophic factor on spinal motoneurons are restricted to fusimotor subtypes. *J. Neurosci.* 28, 2131–2146. doi: 10.1523/JNEUROSCI.5185-07.2008

Goulding, M. (2009). Circuits controlling vertebrate locomotion: moving in a new direction. *Nat. Rev. Neurosci.* 10, 507–518. doi: 10.1038/nrn2608

Goulding, M., Lanuza, G., Sapir, T., and Narayan, S. (2002). The formation of sensorimotor circuits. *Curr. Opin. Neurobiol.* 12, 508–515. doi: 10.1016/S0959-4388(02)00371-9

Goulding, M., and Pfaff, S. L. (2005). Development of circuits that generate simple rhythmic behaviors in vertebrates. *Curr. Opin. Neurobiol.* 15, 14–20. doi: 10.1016/j.conb.2005.01.017

Gowan, K., Helms, A. W., Hunsaker, T. L., Collisson, T., Ebert, P. J., Odom, R., et al. (2001). Crossinhibitory activities of Ngn1 and Math1 allow specification of distinct dorsal interneurons. *Neuron* 31, 219–232. doi: 10.1016/S0896-6273(01)00367-1

Griener, A., Zhang, W., Kao, H., Wagner, C., and Gosgnach, S. (2015). Probing diversity within subpopulations of locomotor-related V0 interneurons. *Dev. Neurobiol*. doi: 10.1002/dneu.22277 [Epub ahead of print].

Grillner, S., and Jessell, T. M. (2009). Measured motion: searching for simplicity in spinal locomotor networks. *Curr. Opin. Neurobiol.* 19, 572–586. doi: 10.1016/j.conb.2009.10.011

Gross, M. K., Dottori, M., and Goulding, M. (2002). Lbx1 specifies somatosensory association interneurons in the dorsal spinal cord. *Neuron* 34, 535–49. doi: 10.1016/S0896-6273(02)00690-6

Gross, M. K., Moran-Rivard, L., Velasquez, T., Nakatsu, M. N., Jagla, K., and Goulding, M. (2000). Lbx1 is required for muscle precursor migration along a lateral pathway into the limb. *Development* 127, 413–424.

Hanson, M. G., and Landmesser, L. T. (2004). Normal patterns of spontaneous activity are required for correct motor axon guidance and the expression of specific guidance molecules. *Neuron* 43, 687–701. doi: 10.1016/j.neuron.2004.08.018

Hegarty, S. V., O'Keeffe, G. W., and Sullivan, A. M. (2013). BMP-Smad 1/5/8 signalling in the development of the nervous system. *Prog. Neurobiol.* 109, 28–41. doi: 10.1016/j.pneurobio.2013.07.002

Helms, A. W., Battiste, J., Henke, R. M., Nakada, Y., Simplicio, N., Guillemot, F., et al. (2005). Sequential roles for Mash1 and Ngn2 in the generation of dorsal spinal cord interneurons. *Development* 132, 2709–2719. doi: 10.1242/dev.01859

Helms, A. W., and Johnson, J. E. (1998). Progenitors of dorsal commissural interneurons are defined by MATH1 expression. *Development* 125, 919–928.

Helms, A. W., and Johnson, J. E. (2003). Specification of dorsal spinal cord interneurons. *Curr. Opin. Neurobiol.* 13, 42–49. doi: 10.1016/S0959-4388(03)00010-2

Henke, R. M., Savage, T. K., Meredith, D. M., Glasgow, S. M., Hori, K., Dumas, J., et al. (2009). Neurog2 is a direct downstream target of the Ptf1a-Rbpj transcription complex in dorsal spinal cord. *Development* 136, 2945–2954. doi: 10.1242/dev.035352

Hinckley, C. A., Hartley, R., Wu, L., Todd, A., and Ziskind-Conhaim, L. (2005). Locomotor-like rhythms in a genetically distinct cluster of interneurons in the mammalian spinal cord. *J. Neurophysiol.* 93, 1439–1449. doi: 10.1152/jn.00647.2004

Hinckley, C. A., and Ziskind-Conhaim, L. (2006). Electrical coupling between locomotor-related excitatory interneurons in the mammalian spinal cord. *J. Neurosci.* 26, 8477–8483. doi: 10.1523/JNEUROSCI.0395-06.2006

Holz, A., Kollmus, H., Ryge, J., Niederkofler, V., Dias, J., Ericson, J., et al. (2010). The transcription factors Nkx2.2 and Nkx2.9 play a novel role in floor plate development and commissural axon guidance. *Development* 137, 4249–4260. doi: 10.1242/dev.053819

Hultborn, H., Jankowska, E., and Lindstrom, S. (1971). Recurrent inhibition of interneurones monosynaptically activated from group Ia afferents. *J. Physiol.* 215, 613–636. doi: 10.1113/jphysiol.1971.sp009488

Hultborn, H., and Udo, M. (1972). Convergence of large muscle spindle (Ia) afferents at interneuronal level in the reciprocal Ia inhibitory pathway to motoneurones. *Acta Physiol. Scand.* 84, 493–499. doi: 10.1111/j.1748-1716.1972.tb05199.x

Jankowska, E. (2001). Spinal interneuronal systems: identification, multifunctional character and reconfigurations in mammals. *J. Physiol.* 533, 31–40. doi: 10.1111/j.1469-7793.2001.0031b.x

Jessell, T. M. (2000). Neuronal specification in the spinal cord: inductive signals and transcriptional codes. *Nat. Rev. Genet.* 1, 20–29. doi: 10.1038/35049541

Ji, S. J., Zhuang, B., Falco, C., Schneider, A., Schuster-Gossler, K., Gossler, A., et al. (2006). Mesodermal and neuronal retinoids regulate the induction and maintenance of limb innervating spinal motor neurons. *Dev. Biol.* 297, 249–261. doi: 10.1016/j.ydbio.2006.05.015

John, A., Wildner, H., and Britsch, S. (2005). The homeodomain transcription factor Gbx1 identifies a subpopulation of late-born GABAergic interneurons in the developing dorsal spinal cord. *Dev. Dyn.* 234, 767–771. doi: 10.1002/dvdy.20568

Jung, H., Lacombe, J., Mazzoni, E. O., Liem, K. F. Jr., Grinstein, J., Mahony, S., et al. (2010). Global control of motor neuron topography mediated by the repressive actions of a single hox gene. *Neuron* 67, 781–796. doi: 10.1016/j.neuron.2010.08.008

Kang, K., Lee, D., Hong, S., Park, S. G., and Song, M. R. (2013). The E3 ligase Mind bomb-1 (Mib1) modulates Delta-Notch signaling to control neurogenesis and gliogenesis in the developing spinal cord. *J. Biol. Chem.* 288, 2580–2592. doi: 10.1074/jbc.M112.398263

Kiehn, O. (2006). Locomotor circuits in the mammalian spinal cord. *Annu. Rev. Neurosci.* 29, 279–306. doi: 10.1146/annurev.neuro.29.051605.112910

Kimura, Y., Okamura, Y., and Higashijima, S. (2006). alx, a zebrafish homolog of Chx10, marks ipsilateral descending excitatory interneurons that participate in the regulation of spinal locomotor circuits. *J. Neurosci.* 26, 5684–5697. doi: 10.1523/JNEUROSCI.4993-05.2006

Kimura, Y., Satou, C., and Higashijima, S. (2008). V2a and V2b neurons are generated by the final divisions of pair-producing progenitors in the zebrafish spinal cord. *Development* 135, 3001–3005. doi: 10.1242/dev.024802

Kriks, S., Lanuza, G. M., Mizuguchi, R., Nakafuku, M., and Goulding, M. (2005). Gsh2 is required for the repression of Ngn1 and specification of dorsal interneuron fate in the spinal cord. *Development* 132, 2991–3002. doi: 10.1242/dev.01878

Kullander, K., Croll, S. D., Zimmer, M., Pan, L., McClain, J., Hughes, V., et al. (2001). Ephrin-B3 is the midline barrier that prevents corticospinal tract axons from recrossing, allowing for unilateral motor control. *Genes Dev.* 15, 877–888. doi: 10.1101/gad.868901

Kwan, A. C., Dietz, S. B., Webb, W. W., and Harris-Warrick, R. M. (2009). Activity of Hb9 interneurons during fictive locomotion in mouse spinal cord. *J. Neurosci.* 29, 11601–11613. doi: 10.1523/JNEUROSCI.1612-09.2009

Ladle, D. R., Pecho-Vrieseling, E., and Arber, S. (2007). Assembly of motor circuits in the spinal cord: driven to function by genetic and experience-dependent mechanisms. *Neuron* 56, 270–283. doi: 10.1016/j.neuron.2007.09.026

Landmesser, L. (1978). The distribution of motoneurones supplying chick hind limb muscles. *J. Physiol.* 284, 371–389. doi: 10.1113/jphysiol.1978.sp012545

Lanuza, G. M., Gosgnach, S., Pierani, A., Jessell, T. M., and Goulding, M. (2004). Genetic identification of spinal interneurons that coordinate left-right locomotor activity necessary for walking movements. *Neuron* 42, 375–386. doi: 10.1016/S0896-6273(04)00249-1

Le Dréau, G., Garcia-Campmany, L., Rabadán, M. A., Ferronha, T., Tozer, S., Briscoe, J., et al. (2012). Canonical BMP7 activity is required for the generation of discrete neuronal populations in the dorsal spinal cord. *Development* 139, 259–268. doi: 10.1242/dev.074948

Lee, K. J., Dietrich, P., and Jessell, T. M. (2000). Genetic ablation reveals that the roof plate is essential for dorsal interneuron specification. *Nature* 403, 734–740. doi: 10.1038/35001507

Lee, K. J., Mendelsohn, M., and Jessell, T. M. (1998). Neuronal patterning by BMPs: a requirement for GDF7 in the generation of a discrete class of commissural interneurons in the mouse spinal cord. *Genes Dev.* 12, 3394–3407. doi: 10.1101/gad.12.21.3394

Lee, S., Lee, B., Joshi, K., Pfaff, S. L., Lee, J. W., and Lee, S. K. (2008). A regulatory network to segregate the identity of neuronal subtypes. *Dev. Cell* 14, 877–889. doi: 10.1016/j.devcel.2008.03.021

Lee, S. K., and Pfaff, S. L. (2001). Transcriptional networks regulating neuronal identity in the developing spinal cord. *Nat. Neurosci.* 4(Suppl.), 1183–1191. doi: 10.1038/nn750

Li, S., Misra, K., and Xiang, M. (2010). A Cre transgenic line for studying V2 neuronal lineages and functions in the spinal cord. *Genesis* 48, 667–672. doi: 10.1002/dvg.20669

Liem, K. F. Jr., Tremml, G., and Jessell, T. M. (1997). A role for the roof plate and its resident TGFbeta-related proteins in neuronal patterning in the dorsal spinal cord. *Cell* 91, 127–138. doi: 10.1016/S0092-8674(01)80015-5

Liem, K. F. Jr., Tremml, G., Roelink, H., and Jessell, T. M. (1995). Dorsal differentiation of neural plate cells induced by BMP-mediated signals from epidermal ectoderm. *Cell* 82, 969–979. doi: 10.1016/0092-8674(95)90276-7

Lin, J. H., Saito, T., Anderson, D. J., Lance-Jones, C., Jessell, T. M., and Arber, S. (1998). Functionally related motor neuron pool and muscle sensory afferent subtypes defined by coordinate ETS gene expression. *Cell* 95, 393–407. doi: 10.1016/S0092-8674(00)81770-5

Liu, J. P., Laufer, E., and Jessell, T. M. (2001). Assigning the positional identity of spinal motor neurons: rostrocaudal patterning of Hox-c expression by FGFs, Gdf11, and retinoids. *Neuron* 32, 997–1012. doi: 10.1016/S0896-6273(01)00544-X

Liu, Z., Hu, X., Huang, C., Zheng, K., Takebayashi, H., Cao, C., et al. (2014). Olig3 is not involved in the ventral patterning of spinal cord. *PLoS ONE* 9:e111076. doi: 10.1371/journal.pone.0111076

Lundfald, L., Restrepo, C. E., Butt, S. J., Peng, C. Y., Droho, S., Endo, T., et al. (2007). Phenotype of V2-derived interneurons and their relationship to the axon guidance molecule EphA4 in the developing mouse spinal cord. *Eur. J. Neurosci.* 26, 2989–3002. doi: 10.1111/j.1460-9568.2007.05906.x

Marion, J. F., Yang, C., Caqueret, A., Boucher, F., and Michaud, J. L. (2005). Sim1 and Sim2 are required for the correct targeting of mammillary body axons. *Development* 132, 5527–5537. doi: 10.1242/dev.02142

Matise, M. P., and Joyner, A. L. (1997). Expression patterns of developmental control genes in normal and Engrailed-1 mutant mouse spinal cord reveal early diversity in developing interneurons. *J. Neurosci.* 17, 7805–7816.

McLean, D. L., Masino, M. A., Koh, I. Y., Lindquist, W. B., and Fetcho, J. R. (2008). Continuous shifts in the active set of spinal interneurons during changes in locomotor speed. *Nat. Neurosci.* 11, 1419–1429. doi: 10.1038/nn.2225

Megason, S. G., and McMahon, A. P. (2002). A mitogen gradient of dorsal midline Wnts organizes growth in the CNS. *Development* 129, 2087–2098.

Mentis, G. Z., Alvarez, F. J., Bonnot, A., Richards, D. S., Gonzalez-Forero, D., Zerda, R., et al. (2005). Noncholinergic excitatory actions of motoneurons in the neonatal mammalian spinal cord. *Proc. Natl. Acad. Sci. U.S.A.* 102, 7344–7349. doi: 10.1073/pnas.0502788102

Meredith, D. M., Masui, T., Swift, G. H., MacDonald, R. J., and Johnson, J. E. (2009). Multiple transcriptional mechanisms control Ptf1a levels during neural development including autoregulation by the PTF1-J complex. *J. Neurosci.* 29, 11139–11148. doi: 10.1523/JNEUROSCI.2303-09.2009

Miesegaes, G. R., Klisch, T. J., Thaller, C., Ahmad, K. A., Atkinson, R. C., and Zoghbi, H. Y. (2009). Identification and subclassification of new Atoh1 derived cell populations during mouse spinal cord development. *Dev. Biol.* 327, 339–351. doi: 10.1016/j.ydbio.2008.12.016

Misra, M., Shah, V., Carpenter, E., McCaffery, P., and Lance-Jones, C. (2009). Restricted patterns of Hoxd10 and Hoxd11 set segmental differences in motoneuron subtype complement in the lumbosacral spinal cord. *Dev. Biol.* 330, 54–72. doi: 10.1016/j.ydbio.2009.03.009

Mizuguchi, R., Kriks, S., Cordes, R., Gossler, A., Ma, Q., and Goulding, M. (2006). Ascl1 and Gsh1/2 control inhibitory and excitatory cell fate in spinal sensory interneurons. *Nat. Neurosci.* 9, 770–778. doi: 10.1038/nn1706

Moran-Rivard, L., Kagawa, T., Saueressig, H., Gross, M. K., Burrill, J., Goulding, M., et al. (2001). Evx1 is a postmitotic determinant of v0 interneuron identity in the spinal cord. *Neuron* 29, 385–399. doi: 10.1016/S0896-6273(01)00213-6

Morikawa, Y., Hisaoka, T., and Senba, E. (2009). Characterization of Foxp2-expressing cells in the developing spinal cord. *Neuroscience* 162, 1150–1162. doi: 10.1016/j.neuroscience.2009.05.022

Muhr, J., Graziano, E., Wilson, S., Jessell, T. M., and Edlund, T. (1999). Convergent inductive signals specify midbrain, hindbrain, and spinal cord identity in gastrula stage chick embryos. *Neuron* 23, 689–702. doi: 10.1016/S0896-6273(01)80028-3

Muller, T., Anlag, K., Wildner, H., Britsch, S., Treier, M., and Birchmeier, C. (2005). The bHLH factor Olig3 coordinates the specification of dorsal neurons in the spinal cord. *Genes Dev.* 19, 733–743. doi: 10.1101/gad.326105

Muller, T., Brohmann, H., Pierani, A., Heppenstall, P. A., Lewin, G. R., et al. (2002). The homeodomain factor lbx1 distinguishes two major programs of neuronal differentiation in the dorsal spinal cord. *Neuron* 34, 551–562. doi: 10.1016/S0896-6273(02)00689-X

Muroyama, Y., Fujihara, M., Ikeya, M., Kondoh, H., and Takada, S. (2002). Wnt signaling plays an essential role in neuronal specification of the dorsal spinal cord. *Genes Dev.* 16, 548–553. doi: 10.1101/gad.937102

Myers, C. P., Lewcock, J. W., Hanson, M. G., Gosgnach, S., Aimone, J. B., Gage, H. F., et al. (2005). Cholinergic input is required during embryonic development to mediate proper assembly of spinal locomotor circuits. *Neuron* 46, 37–49. doi: 10.1016/j.neuron.2005.02.022

Nornes, H. O., and Carry, M. (1978). Neurogenesis in spinal cord of mouse: an autoradiographic analysis. *Brain Res.* 159, 1–6. doi: 10.1016/0006-8993(78)90105-1

Novitch, B. G., Chen, A. I., and Jessell, T. M. (2001). Coordinate regulation of motor neuron subtype identity and pan-neuronal properties by the bHLH repressor Olig2. *Neuron* 31, 773–789. doi: 10.1016/S0896-6273(01)00407-X

Okigawa, S., Mizoguchi, T., Okano, M., Tanaka, H., Isoda, M., Isoda, M., Jiang, Y. J., et al. (2014). Different combinations of Notch ligands and receptors regulate V2 interneuron progenitor proliferation and V2a/V2b cell fate determination. *Dev. Biol.* 391, 196–206. doi: 10.1016/j.ydbio.2014.04.011

Panayi, H., Panayiotou, E., Orford, M., Genethliou, N., Mean, R., Lapathitis, G., et al. (2010). Sox1 is required for the specification of a novel p2-derived interneuron subtype in the mouse ventral spinal cord. *J. Neurosci.* 30, 12274–12280. doi: 10.1523/JNEUROSCI.2402-10.2010

Peng, C. Y., Yajima, H., Burns, C. E., Zon, L. I., Sisodia, S. S., Pfaff, S. L., et al. (2007). Notch and MAML signaling drives Scl-dependent interneuron diversity in the spinal cord. *Neuron* 53, 813–827. doi: 10.1016/j.neuron.2007.02.019

Pfaff, S. L., Mendelsohn, M., Stewart, C. L., Edlund, T., and Jessell, T. M. (1996). Requirement for LIM homeobox gene Isl1 in motor neuron generation reveals a motor neuron-dependent step in interneuron differentiation. *Cell* 84, 309–320. doi: 10.1016/S0092-8674(00)80985-X

Pierani, A., Brenner-Morton, S., Chiang, C., and Jessell, T. M. (1999). A sonic hedgehog-independent, retinoid-activated pathway of neurogenesis in the ventral spinal cord. *Cell* 97, 903–915. doi: 10.1016/S0092-8674(00)80802-8

Pierani, A., Moran-Rivard, L., Sunshine, M. J., Littman, D. R., Goulding, M., and Jessell, T. M. (2001). Control of interneuron fate in the developing spinal

cord by the progenitor homeodomain protein Dbx1. *Neuron* 29, 367–384. doi: 10.1016/S0896-6273(01)00212-4

Pillai, A., Mansouri, A., Behringer, R., Westphal, H., and Goulding, M. (2007). Lhx1 and Lhx5 maintain the inhibitory-neurotransmitter status of interneurons in the dorsal spinal cord. *Development* 134, 357–366. doi: 10.1242/dev.02717

Qian, Y., Shirasawa, S., Chen, C. L., Cheng, L., and Ma, Q. (2002). Proper development of relay somatic sensory neurons and D2/D4 interneurons requires homeobox genes Rnx/Tlx-3 and Tlx-1. *Genes Dev.* 16, 1220–1233. doi: 10.1101/gad.982802

Quiroga, A. C., Stolt, C. C., Del Corral, R. D., Dimitrov, S., Perez-Alcala, S., Sock, E., et al. (2015). Sox5 controls dorsal progenitor and interneuron specification in the spinal cord. *Dev. Neurobiol.* 75, 522–538. doi: 10.1002/dneu.22240

Ramos, C., Rocha, S., Gaspar, C., and Henrique, D. (2010). Two Notch ligands, Dll1 and Jag1, are differently restricted in their range of action to control neurogenesis in the mammalian spinal cord. *PLoS ONE* 5:e15515. doi: 10.1371/journal.pone.0015515

Richards, D. S., Griffith, R. W., Romer, S. H., and Alvarez, F. J. (2014). Motor axon synapses on renshaw cells contain higher levels of aspartate than glutamate. *PLoS ONE* 9:e97240. doi: 10.1371/journal.pone.0097240

Roelink, H., Augsburger, A., Heemskerk, J., Korzh, V., Norlin, S., Ruiz i Altaba, A., et al. (1994). Floor plate and motor neuron induction by vhh-1, a vertebrate homolog of hedgehog expressed by the notochord. *Cell* 76, 761–775. doi: 10.1016/0092-8674(94)90514-2

Sander, M., Paydar, S., Ericson, J., Briscoe, J., Berber, E., German, M., et al. (2000). Ventral neural patterning by Nkx homeobox genes: Nkx6.1 controls somatic motor neuron and ventral interneuron fates. *Genes Dev.* 14, 2134–2139. doi: 10.1101/gad.820400

Sapir, T., Geiman, E. J., Wang, Z., Velasquez, T., Mitsui, S., Yoshihara, Y., et al. (2004). Pax6 and engrailed 1 regulate two distinct aspects of renshaw cell development. *J. Neurosci.* 24, 1255–1264. doi: 10.1523/JNEUROSCI.3187-03.2004

Saueressig, H., Burrill, J., and Goulding, M. (1999). Engrailed-1 and netrin-1 regulate axon pathfinding by association interneurons that project to motor neurons. *Development* 126, 4201–4212.

Scardigli, R., Schuurmans, C., Gradwohl, G., and Guillemot, F. (2001). Crossregulation between Neurogenin2 and pathways specifying neuronal identity in the spinal cord. *Neuron* 31, 203–217. doi: 10.1016/S0896-6273(01)00358-0

Sharma, K., Leonard, A. E., Lettieri, K., and Pfaff, S. L. (2000). Genetic and epigenetic mechanisms contribute to motor neuron pathfinding. *Nature* 406, 515–519. doi: 10.1038/35020078

Sharma, K., Sheng, H. Z., Lettieri, K., Li, H., Karavanov, A., Potter, S., et al. (1998). LIM homeodomain factors Lhx3 and Lhx4 assign subtype identities for motor neurons. *Cell* 95, 817–828. doi: 10.1016/S0092-8674(00)81704-3

Shirasaki, R., and Pfaff, S. L. (2002). Transcriptional codes and the control of neuronal identity. *Annu. Rev. Neurosci.* 25, 251–281. doi: 10.1146/annurev.neuro.25.112701.142916

Shneider, N. A., Brown, M. N., Smith, C. A., Pickel, J., and Alvarez, F. J. (2009). Gamma motor neurons express distinct genetic markers at birth and require muscle spindle-derived GDNF for postnatal survival. *Neural Dev.* 4, 42. doi: 10.1186/1749-8104-4-42

Skaggs, K., Martin, D. M., and Novitch, B. G. (2011). Regulation of spinal interneuron development by the Olig-related protein Bhlhb5 and Notch signaling. *Development* 138, 3199–3211. doi: 10.1242/dev.057281

Sockanathan, S., and Jessell, T. M. (1998). Motor neuron-derived retinoid signaling specifies the subtype identity of spinal motor neurons. *Cell* 94, 503–514. doi: 10.1016/S0092-8674(00)81591-3

Sockanathan, S., Perlmann, T., and Jessell, T. M. (2003). Retinoid receptor signaling in postmitotic motor neurons regulates rostrocaudal positional identity and axonal projection pattern. *Neuron* 40, 97–111. doi: 10.1016/S0896-6273(03)00532-4

Stam, F. J., Hendricks, T. J., Zhang, J., Geiman, E. J., Francius, C., Labosky, P. A., et al. (2012). Renshaw cell interneuron specialization is controlled by a temporally restricted transcription factor program. *Development* 139, 179–190. doi: 10.1242/dev.071134

Stepien, A. E., and Arber, S. (2008). Probing the locomotor conundrum: descending the 'V' interneuron ladder. *Neuron* 60, 1–4. doi: 10.1016/j.neuron.2008.09.030

Stepien, A. E., Tripodi, M., and Arber, S. (2010). Monosynaptic rabies virus reveals premotor network organization and synaptic specificity of cholinergic partition cells. *Neuron* 68, 456–472. doi: 10.1016/j.neuron.2010.10.019

Tanabe, Y., William, C., and Jessell, T. M. (1998). Specification of motor neuron identity by the MNR2 homeodomain protein. *Cell* 95, 67–80. doi: 10.1016/S0092-8674(00)81783-3

Thaler, J., Harrison, K., Sharma, K., Lettieri, K., Kehrl, J., and Pfaff, S. L. (1999). Active suppression of interneuron programs within developing motor neurons revealed by analysis of homeodomain factor HB9. *Neuron* 23, 675–687. doi: 10.1016/S0896-6273(01)80027-1

Thaler, J. P., Koo, S. J., Kania, A., Lettieri, K., Andrews, S., Cox, C., et al. (2004). A postmitotic role for Isl-class LIM homeodomain proteins in the assignment of visceral spinal motor neuron identity. *Neuron* 41, 337–350. doi: 10.1016/S0896-6273(04)00011-X

Thaler, J. P., Lee, S. K., Jurata, L. W., Gill, G. N., and Pfaff, S. L. (2002). LIM factor Lhx3 contributes to the specification of motor neuron and interneuron identity through cell-type-specific protein-protein interactions. *Cell* 110, 237–249. doi: 10.1016/S0092-8674(02)00823-1

Timmer, J. R., Wang, C., and Niswander, L. (2002). BMP signaling patterns the dorsal and intermediate neural tube via regulation of homeobox and helix-loop-helix transcription factors. *Development* 129, 2459–2472.

Tracey, D. P. (1985). "The somatosensory system." in *The Rat Nervous System*, ed. G. Paxinos (San Diego, CA: Academic Press), 129–152.

Tsuchida, T., Ensini, M., Morton, S. B., Baldassare, M., Edlund, T., Jessell, T. M., et al. (1994). Topographic organization of embryonic motor neurons defined by expression of LIM homeobox genes. *Cell* 79, 957–970. doi: 10.1016/0092-8674(94)90027-2

Vallstedt, A., and Kullander, K. (2013). Dorsally derived spinal interneurons in locomotor circuits. *Ann. N. Y. Acad. Sci.* 1279, 32–42. doi: 10.1111/j.1749-6632.2012.06801.x

Vallstedt, A., Muhr, J., Pattyn, A., Pierani, A., Mendelsohn, M., Sander, M., et al. (2001). Different levels of repressor activity assign redundant and specific roles to Nkx6 genes in motor neuron and interneuron specification. *Neuron* 31, 743–755. doi: 10.1016/S0896-6273(01)00412-3

Wang, Z., Li, L., Goulding, M., and Frank, E. (2008). Early postnatal development of reciprocal Ia inhibition in the murine spinal cord. *J. Neurophysiol.* 100, 185–196. doi: 10.1152/jn.90354.2008

Watterson, R. L. (1965). *Organogenesis*. New York, NY: Holt, Rinehart and Winston, 129–159.

Wenner, P., Matise, M. P., Joyner, A., and O'Donovan, M. J. (1998). Physiological and molecular characterization of interneurons in the developing spinal cord. *Ann. N. Y. Acad. Sci.* 860, 425–427. doi: 10.1111/j.1749-6632.1998.tb09066.x

Wenner, P., O'Donovan, M. J., and Matise, M. P. (2000). Topographical and physiological characterization of interneurons that express engrailed-1 in the embryonic chick spinal cord. *J. Neurophysiol.* 84, 2651–2657.

Wichterle, H., Lieberam, I., Porter, J. A., and Jessell, T. M. (2002). Directed differentiation of embryonic stem cells into motor neurons. *Cell* 110, 385–397. doi: 10.1016/S0092-8674(02)00835-8

Wickersham, I. R., Lyon, D. C., Barnard, R. J., Mori, T., Finke, S., Conzelmann, K. K., et al. (2007). Monosynaptic restriction of transsynaptic tracing from single, genetically targeted neurons. *Neuron* 53, 639–647. doi: 10.1016/j.neuron.2007.01.033

Wilson, J. M., Hartley, R., Maxwell, D. J., Todd, A. J., Lieberam, I., Kaltschmidt, J. A., et al. (2005). Conditional rhythmicity of ventral spinal interneurons defined by expression of the Hb9 homeodomain protein. *J. Neurosci.* 25, 5710–5719. doi: 10.1523/JNEUROSCI.0274-05.2005

Wilson, S. I., Shafer, B., Lee, K. J., and Dodd, J. (2008). A molecular program for contralateral trajectory: Rig-1 control by LIM homeodomain transcription factors. *Neuron* 59, 413–424. doi: 10.1016/j.neuron.2008.07.020

Yang, X., Tomita, T., Wines-Samuelson, M., Beglopoulos, V., Tansey, M. G., Kopan, R., et al. (2006). Notch1 signaling influences v2 interneuron and motor neuron development in the spinal cord. *Dev. Neurosci.* 28, 102–117. doi: 10.1159/000090757

Yokoyama, N., Romero, M. I., Cowan, C. A., Galvan, P., Helmbacher, F., Charnay, P., et al. (2001). Forward signaling mediated by ephrin-B3 prevents contralateral corticospinal axons from recrossing the spinal cord midline. *Neuron* 29, 85–97. doi: 10.1016/S0896-6273(01)00182-9

Zagoraiou, L., Akay, T., Martin, J. F., Brownstone, R. M., Jessell, T. M., and Miles, G. B. (2009). A cluster of cholinergic premotor interneurons modulates mouse locomotor activity. *Neuron* 64, 645–662. doi: 10.1016/j.neuron.2009.10.017

Zhang, J., Lanuza, G. M., Britz, O., Wang, Z., Siembab, V. C., Zhang, Y., et al. (2014). V1 and v2b interneurons secure the alternating flexor-extensor motor activity mice require for limbed locomotion. *Neuron* 82, 138–150. doi: 10.1016/j.neuron.2014.02.013

Zhang, Y., Narayan, S., Geiman, E., Lanuza, G. M., Velasquez, T., Shanks, B., et al. (2008). V3 spinal neurons establish a robust and balanced locomotor rhythm during walking. *Neuron* 60, 84–96. doi: 10.1016/j.neuron.2008.09.027

Zhong, G., Droho, S., Crone, S. A., Dietz, S., Kwan, A. C., Kwan, A. C., et al. (2010). Electrophysiological characterization of V2a interneurons and their locomotor-related activity in the neonatal mouse spinal cord. *J. Neurosci.* 30, 170–182. doi: 10.1523/JNEUROSCI.4849-09.2010

Zou, M., Luo, H., and Xiang, M. (2015). Selective neuronal lineages derived from Dll4-expressing progenitors/precursors in the retina and spinal cord. *Dev. Dyn.* 244, 86–97. doi: 10.1002/dvdy.24185

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

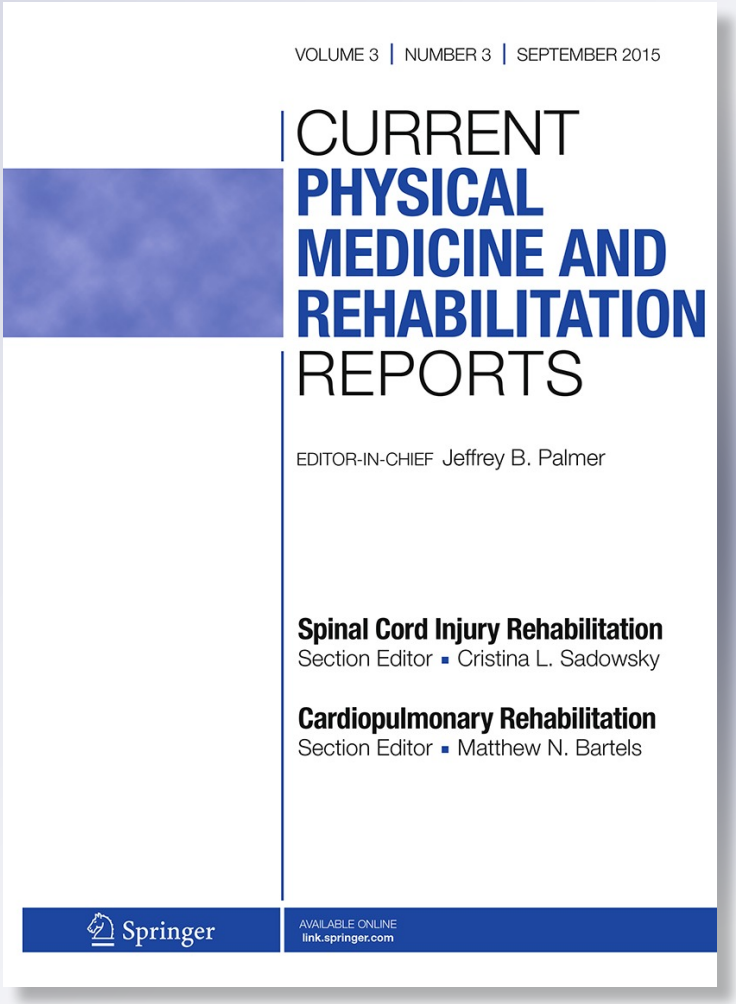
Strategies and lessons in spinal cord injury rehabilitation

Tianyi Niu, William A. Alaynick & Daniel C. Lu

Current Physical Medicine and Rehabilitation Reports

e-ISSN 2167-4833
Volume 3
Number 3

Curr Phys Med Rehabil Rep (2015)
3:206-213
DOI 10.1007/s40141-015-0096-z



Springer

Curr Phys Med Rehabil Rep (2015) 3:206–213
DOI 10.1007/s40141-015-0096-z

Strategies and lessons in spinal cord injury rehabilitation

Tianyi Niu[1] · **William A. Alaynick**[1] · **Daniel C. Lu**[1]

Published online: 22 July 2015

Abstract The spinal cord is often underappreciated as part of the central nervous system. Like the brain, the spinal cord can independently carry out relatively complex behaviors, such as left–right and flexor–extension alternation of the limbs, through activation of resident central pattern generator circuitry. Here the spinal cord integrates ascending or local proprioceptive information with descending sensory and volitional information. In the context of injury, portions of the isolated spinal cord may still be capable of carrying out sophisticated processing for sensorimotor function. Several modes of stimulation appear to activate the central pattern generating circuitry in SCI: treadmill stepping, magnetic stimulation, electrical stimulation, vibratory stimulation, and pharmacologic agents. Like the brain, the spinal cord is capable of classical and operant conditioning. This observation highlights the need for well-planned therapeutic interventions that work with the innate behavior of the cord and avoid maladaptive learning that can occur if noxious stimuli are present during rehabilitation. Patient-specific multimodal therapies that work with innate spinal cord behaviors are most likely to benefit patients with SCI.

Keywords Spinal cord injury · Rehabilitation · Electrical stimulation · Magnetic stimulation · Treadmill stepping · Operant conditioning

Introduction

The central nervous system consists of the spinal cord, hindbrain, midbrain, and forebrain. The spinal cord contains 31 segments that mediate all motor and sensory functions (save those of the cranial nerves). The spinal cord does not simple relay these descending commands and ascending sensory signals. In addition to substantial fibers of passage that surround the cord with white matter, the central region of the cord contains gray matter. This gray matter contains dozens of distinct interneuron cell types, as well as the pools of motor neurons that are distinct for each muscle of the body [1, 2•].

The majority of spinal cord injuries happen at one location affecting one, or a few, spinal levels. As a result, a great deal of spinal cord tissue is unharmed—although it may be isolated from the brain—unable to convey ascending sensory signals or receive descending motor commands. The field of rehabilitation for spinal cord injury has developed, and demonstrated, the concept that spared spinal cord can be activated by several means to restore function. The combination of activation modalities, and in what context they are delivered, appears to be critical to the appropriate re-activation of spinal cord following injury. Below we review key concepts in the field of spinal cord injury rehabilitation.

This article is part of the Topical Collection on *Spinal Cord Injury Rehabilitation*.

✉ Daniel C. Lu
DCLu@mednet.ucla.edu

Tianyi Niu
TNiu@mednet.ucla.edu

William A. Alaynick
WAlaynick@mednet.ucla.edu

[1] Department of Neurosurgery, David Geffen School of Medicine, University of California, Los Angeles, 650 Charles E. Young Drive, Wasserman Building, Room 562, Los Angeles, CA 90095, USA

Basis of human locomotion

Coordination of the limbs during locomotion, or during stereotyped behaviors, is mediated by several interdependent regions of the central nervous system, including the spinal cord. The recognition that the spinal cord can semi-autonomously coordinate limb movement was proposed by Graham-Brown in 1911 with his half-center hypothesis to explain the observation that reduced preparations can produce coordinated movement [3]. This early model, developed in cats, did not account for descending or sensory information. Over 70 years later, work in isolated lamprey spinal cords resulted in the concept of Central Pattern Generators (CPGs) [4]. Within the lumbosacral cord of model mammals, cat, rat, and mouse, lesion and isolated cord experiments have demonstrated that left–right and flexor–extensor coordination of the hindlimbs can be induced (in actual or fictive locomotion) by pharmacologic or electrical stimulation, or by sensory stimulation [5]. With the advent of molecular genetic experiments in mice, several interneuron classes have been identified that contribute to aspects of left–right and flexor–extensor coordination, and rates of CPG activity [1, 6, 2•].

These reductionist experiments have been useful in solidifying the CPG model; however, rehabilitation requires that complex peripheral proprioceptive (such as walking over varied terrain), central unconscious sensory (vestibular, auditory, visual), and volitional (direction, speed) information to seamlessly modulate the intrinsic CPG circuitry [7]. It appears that CPGs are cyclically permissive in integrating or restrictive in gating these information streams depending on the behavioral state of the animal [8, 9]. While the CPG has robust self-regulation, it can incorporate descending tone [10] from primary and supplementary motor cortex during locomotion [11, 12] and in proportion to walking speed [13].

Activation of lumbosacral CPG circuitry, such as by electrically stimulating the tibial nerve, results in coordination of the upper/fore with the lower/hind limbs via propriospinal connections [14, 15]. As such, leg locomotion alters the reflex behavior in proximal arm muscles, revealing task-dependent coupling of these upper and lower limb spinal circuitries. This status-dependent interaction of distributed motor systems highlights the importance of designing rehabilitation schemes that work within the innate permissive "up states" or resistive "down states" to external modulation through ascending or descending inputs.

Plasticity with locomotor training

Learning and plasticity in the spinal cord is demonstrated by the observation that animals lacking motor input to the hind/lower limbs can learn to stand and walk on a treadmill without input from the brain [16–18], including human subjects [19]. Here the rehabilitative task is well matched to the innate behavior of the CPG: alternating flexion–extension and left–right alternation [20, 21]. This results in a useful and adaptive behavioral response by the spinal circuitry and CPG. Care must be taken by healthcare professionals to be assured that the rehabilitative strategy is appropriate to the natural behavior of the task being trained, as well and the receptivity status of the cord.

Spinal cord injuries in clinical settings may occur with additional trauma within dermatomes and myotomes innervated by spinal segments below the level of injury. Rehabilitative strategies that are performed in the presence of pain signaling to the isolated cord level(s) may result in adaptive or maladaptive spinal cord learning. For instance, a form of Pavlovian leaning, where a behavior (e.g., salivation) can be elicited with a conditioned stimulus (e.g., a bell) that has been paired with and unconditioned stimulus (e.g., food) that can be found within the spinal cord. In spinal animals, it has been shown that innocuous thigh stimulation can be paired with a noxious plantar stimulation. As a result, the conditioned thigh stimulus can produce a paw withdrawal without the unconditioned noxious stimulus [22, 23]. Here, as in the treadmill training above, the learned behavioral response is appropriate to the environmental conditions and the avoidance of pain. Care must be taken to avoid inadvertent, potentially maladaptive, Pavlovian conditioning of isolated spinal segments during rehabilitation.

In similar experiments in spinalized rats, animals can be trained to flex their foot to avoid a shock when the foot is lowered [24]. This context-appropriate behavior improves recovery from spinal cord injury. Importantly, if, however, the stimulus is delivered at random and without consideration of the limb position, a spinal learning deficit with tactile hyperreactivity and impaired recovery of function is observed [25]. It is therefore likely that rehabilitative strategies that elicit limb pain in a random manner or regardless of limb position, even in sensory complete individuals, could elicit maladaptive spinal learning.

Locomotor ability after SCI

Most SCIs result from trauma and majority of those injuries result in incomplete loss of motor and sensory function with some residual functions below the level of injury [26]. This indicates that there are some intact functional neuronal connections across the injury level. Those connections, if activated, may contribute to the plasticity of the nervous system and enhance the potential of functional recovery after SCI [27]. In cases of complete SCI where there is no function below the level of injury, the functional recovery rests heavily on the plasticity of resident spinal locomotor circuitry below the level of injury [28]. The

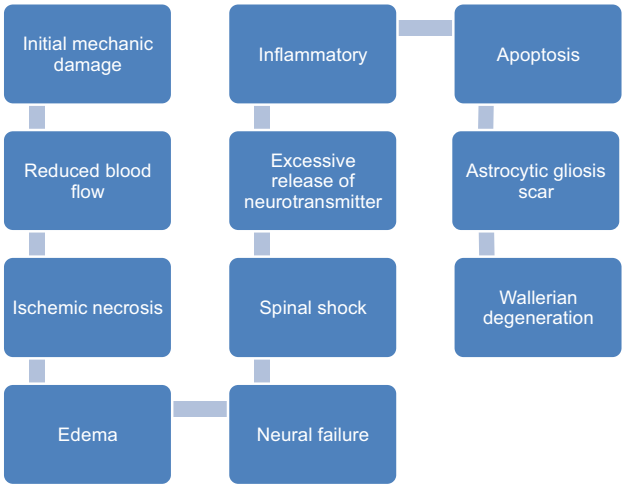


Fig. 1 Sequence of physiologic events following SCI. In SCI, there is the initial insult resulting often from mechanical trauma, such as the shearing force of displaced vertebral bodies. This initial trauma disrupts the vasculature or compresses tissue preventing normal blood flow. Tissue that has been deprived of blood flow for sufficient duration will undergo ischemic necrosis. This is followed by edema due to increased capillary permeability. Neurons in the affected regions that are not killed are exposed to milieu that is not conducive to normal function and undergo neural failure. As a result of these conditions, an acute period of spinal shock lasting for days or weeks can produce a transient loss of movement and sensation. During this period of spinal shock, excessive neurotransmitter may be released, perhaps in an attempt by the local neurons to re-establish homeostasis. Excessive neuronal activity and the necrosis of cells due to ischemia result in inflammation. Inflammation results in apoptotic death of cells in and within the penumbra of the affected region. Over several months, inappropriately a large number of glia are recruited to the region. As a result, an astrocytic glial scar can form that deposits excess myelin and other molecules that inhibit axon growth. Neurons with soma that are outside the focus of the injury or the penumbra may still have damaged axons within the injured region. Neurons with severed axons will slowly retract their axons and die in a characteristic manner called Wallerian degeneration

basis of any functional recovery after SCI is the idea that the central nervous system is a dynamic system that constantly changes, adapts, and reorganizes, particularly after an injury. Bareyre and Schwab summarized the order of events following SCI: from swelling to excess neurotransmitter release to gliosis to eventual scarring and Wallerian degeneration [29] (Fig. 1).

It has been well documented in both mammalian [30] and rodent models [31] that following severe SCI, a significant proportion of locomotor function is maintained by spinal central pattern generator circuitry that generates oscillatory patterns in response to stimulation, such as propriospinal feedback from the limbs. In fact, experimental neonatal rats with complete transection of the spinal cord are still capable of performing treadmill locomotion [32]. Corticospinal tracts, along with the proprioceptive afferent signals from the muscles and extremities, appear to play a more prominent role in modulating and fine tuning of the otherwise crude locomotion and therefore are much more important in skilled movements [33]. This is especially true in humans, unlike rodents, where corticospinal projections synapse directly on motor neurons.

Electrophysiology studies reveal that the amplitudes of locomotor EMG signals and polysynaptic spinal reflexes increase slowly over time as patients recover from SCI. These plateau after a few months [34] at an EMG activity level that is significantly lower than a healthy individual [35]. Clinically, the patient exhibits increased spinal reflexes, increased muscle tone, and spasticity. In SCI individuals, after some minutes of assisted locomotion, the EMG amplitude decreases to background noise level. This is termed "EMG exhaustion" by Hubli et al. [36••]. This characteristic change is unique to humans, as so far, there are no animal model conditions that can recreate this phenomenon. The phenomenon appears to be closely related to immobility rather than the quality of the injury, as it is evident that incomplete SCI patients who are wheelchair bound show same exhaustion as patients with complete SCI; while patients who undergo frequent locomotion activities show no such exhaustion [37]. The exact cause of the exhaustion is unknown at this time. One theory is that SCI results in reduced excitatory, or increased inhibitory, input from supraspinal sources and afferent input from peripheral sources, and thus an overall increase in inhibitory influence of the locomotor circuitry [37]. This theory, if true, offers a therapeutic opportunity to enhance functions through blocking of inhibitory signals (or improving excitatory signals).

Effect of locomotor training after SCI

As mentioned above, after SCI, with neuronal plasticity, there is a spontaneous re-organization of cortical sensory and motor representations, demonstrating substantial CNS plasticity, particularly in incomplete subjects. In addition to this, however, there is also re-organization and re-structuring of the spinal circuitry after locomotor training [38]. One of the most common forms of locomotor training is body weight supported treadmill training (BWSTT). During the training, the patient's body weight is supported by a harness or a robotic apparatus. The subject is then asked to step on a motorized treadmill. In complete SCI subjects, steps can also be assisted by therapists or assistive robotics. In both animal and human models, there are numerous studies that support BWSTT as a method to restore locomotion.

In mice model, BWSTT increases axonal sprouting proximal to the injury level [39] and the expression of neurotrophic factors in the spinal cord [40, 41]. In human subjects, functional MRI evidence suggests that BWSTT induces greater activation of bilateral cortical and cerebellar sensory, and motor areas [42]. That this may be a general phenomenon of recovery is reflected in the

observation that similar changes are seen in post-stroke patients after BWSTT training [43]. Electrophysiological evidence shows that BWSTT increases MEP amplitude, reorganizes and re-establishes cortical control of spinal reflexes [44], and spinal interneuron afferents [45].

Despite the above promising findings, a recent Cochrane review by Mehrholz showed that there is no statistically significant effect of locomotor training on walking function in human subjects after SCI, when comparing BWSTT, with or without functional electrical stimulation, or robotic-assisted locomotor training. However, as pointed out by Mehrholz et al., the Cochrane review is limited in its utility because there are only four randomized control trials available for comparison [46••].

Importance of sensory/proprioceptive cues

Following an injury to the spinal cord that results in a reduction in spinal neural and motor activity [47], there is an attendant reduction in the amount of proprioceptive sensory information generated and fed into the spinal cord. Rehabilitative strategies have sought to replace this deficit in proprioceptive feedback onto spinal and CPG circuitries by using vibratory stimulation of the quadriceps and hamstring muscles [48], epidural stimulation of the dorsal cord [19], electrical stimulation of the sural or peroneal nerves [49], and TMS stimulation of the cord [50]. These approaches may create a condition where the CPG circuitry is modulated such that it is in a permissive state to allow incorporation of sensory input.

Work in cats and rodents have demonstrated that a multimodal method of modulation is most effective. Here rehabilitative strategies have used treadmill training, electrical stimulation of the spinal cord, and the use of serotonergic and noradrenergic agonists [51–54]. While limited evidence has been published regarding the use of pharmacology for the recovery of motor function in human subjects, other modalities are being attempted [55]. Other methods, such as transcutaneous spinal direct current stimulation (tsDCS) [56] and the use of paired spinal associative stimulations of H-reflexes and transcranial magnetic stimulation [57], are being applied in the hopes of producing a permissive spinal cord state and behaviors appropriate to the natural function of the spinal cord.

Novel treatments: modulation of spinal cord excitability

Experimental studies in spinalized animals confirm the importance of afferent information in modulating locomotion [51, 52]. The functional state of the spinal cord is highly dependent on afferent sensory information [58]. And it has been argued that after a severe SCI, due to loss of sensory input, the functional state of the spinal cord is depressed and this further negatively impacts locomotor recovery [36••]. Therefore, tools to activate the locomotor circuitry after SCI may be useful in restoration of locomotor function. Approaches include, body loading (by body weight support treadmill apparatus) [59•], vibratory stimuli of the muscles [60], electrical stimulation of the peripheral nerves [61], electrical [62] and magnetic [50] stimulation of the spinal cord, and pharmacological agents [51, 52] that activate the spinal locomotor circuitry.

Body weight support with manually or robotically assisted devices provides sensory feedback to the cord and is often used to activate the spinal locomotor circuitry [61]. During axial loading, load information from joint and muscles, and tactile sensory information from foot, are thought to be integrated and influence the locomotor central pattern generator to adapt to changing terrain. The effect of this strategy in activating the excitability of the spinal cord circuitry has been demonstrated by an associated increase in H-reflex and data suggesting that functional recovery can be realized in incomplete and complete SCI patients [59•].

Vibratory stimuli at the muscle and joint serve to activate predominantly Ia afferent fibers (muscle spindles) and to lesser extent 1b fibers (Golgi tendon organs) [60] and have the capacity of imparting kinesthesia—sense and positional movement of extremity—in a non-injured subject. Additionally, the vibrated muscle can generate activity that can induce step-like movement in normal and incomplete SCI subject [63•]. Electrical stimulation of peripheral nerves, which may recapitulate some aspects of vibratory stimuli, can modulate the functional state of the CPG. Specific stimulation of the sural of peroneal nerves (0.3 ms pulse width, 2–3 mA, 60 Hz), can induce air-stepping in a gravity-eliminating apparatus that can be enhanced with vibratory muscle stimuli, in healthy subjects [49].

Spinal cord modulation by electrical and magnetic energy is thought to enhance the activation state of the spinal cord such that residual supraspinal input is unmasked and voluntary function revealed (reviewed in [64] Fig. 2), hypothesized to re-enable lower extremity function after severe SCI [65]. Non-invasive means of delivering magnetic (via magnetic coil; [50] and electrical (via surface electrodes; [66•]) stimulation have also been demonstrated to facilitate rhythmic locomotor-like activity in normal subjects. Whether these methods can impact locomotor function in SCI awaits further investigation.

Activation of the spinal cord locomotor circuitry in animals can be performed with pharmacological agents, particularly serotonergic and noradrenergic agonists. However, there is limited clinical evidence that pharmacological treatment can improve locomotor function after SCI [55].

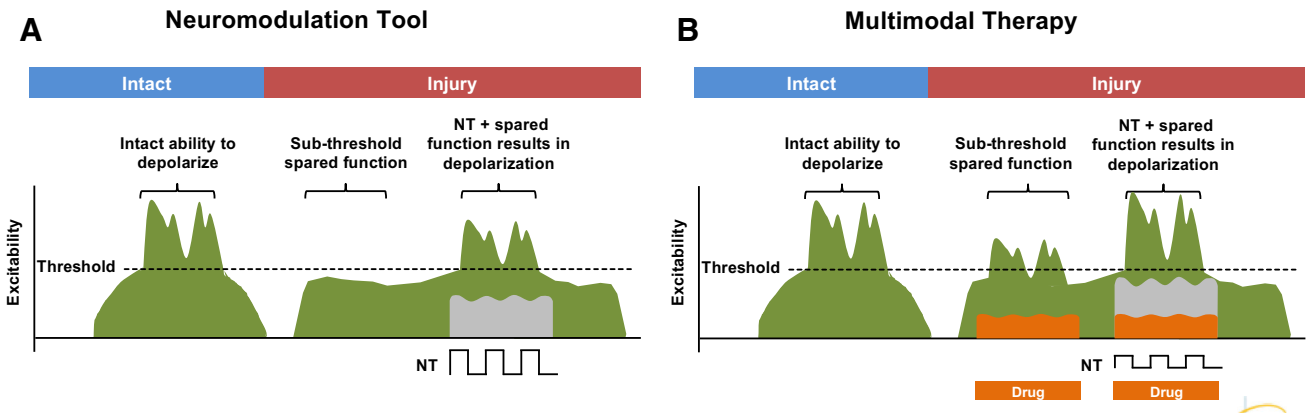


Fig. 2 **a** Neuromodulatory Interventions in SCI. In the intact cord, there is sufficient ascending, local, and descending input to effect depolarization of motor neurons to produce movement. In the context of injury, however, the reduction in inputs to the areas of the spinal cord below the injury results in a state that does not reach the threshold and cannot depolarize motor neurons resulting in paralysis. The addition of electrical or magnetic stimulation helps increase the state of the spinal cord such that previously subthreshold inputs can now depolarize some motor neurons to produce movement. **b** Rehabilitative strategies that employ interventions with mechanistically discreet actions might result in additive or synergistic improvements in function to recruit greater numbers of motor neurons

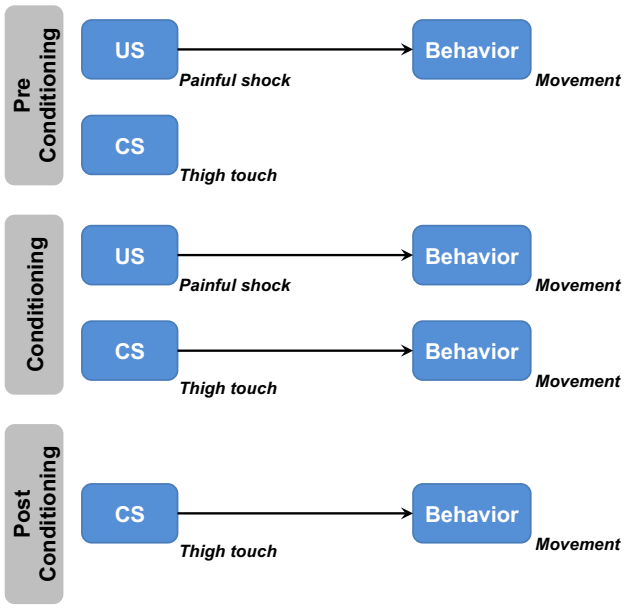


Fig. 3 The spinal cord is capable of learning. Preconditioning. In Pavlovian classical conditioning, the Unconditioned Stimulus, US, is an innate, unlearned behavior, such as retracting the limb from a painful shock. A stimulus for which there is no innate behavior, such as light touch on the thigh, does not result in any innate behavior, and the animal or in the case isolated spinal cord, can be conditioned to respond. Conditioning. Here the Conditioned Stimulus, CS, is paired (delivered at the same time) as the US. This is done over multiple learning sessions to establish the conditioning. Post Conditioning. Once conditioning has been established, the CS will evoke the same behavior as the US. In the context of spinal cord rehabilitation, care must be taken to avoid inadvertently painful stimulation even in sensory complete individuals. Maladaptive learning could occur with pairing of tasks or stimuli meant to improve movement if they co-occur with or produce pain, respectively

There are currently no published studies that assess the utility of pharmacological treatment in patients with complete spinal cord injury (ASIA Impairment Scale A) to determine whether treatment can unmask residual lower extremity function. Such an approach may be fruitful as interventions that enhance spinal cord activity state may serve to enable attenuated supraspinal volitional input distal to lesion of injury (Fig. 3). Additionally, there are currently no multimodal studies that combine electrical/magnetic forms of neuromodulation with pharmacological means to further activate the spinal cord, which may be useful.

Changes in other systems

While there may be limited functional recovery after locomotor training, numerous changes in other systems have been well documented. For example, locomotor training has been shown to increase muscle and bone mass [67, 68]. It has also been shown to increase heart rate, blood pressure regulation, and reduce ventilatory need [69–71]. And perhaps, most importantly, many studies have shown significant increased subjective sense of independence in SCI patients after locomotor training [72, 73].

Conclusion

Because the spinal cord can carry our semi-independent behaviors and is capable of learning, it is critical to design rehabilitative strategies that work with the innate behaviors

of the cord. The innate behavior of integrating proprioceptive information from the limbs and body axis in order to modulate the central pattern generating circuitry is one that shows great promise. Several methods of increasing proprioceptive input (treadmill with body weight support and vibratory stimuli of muscle proprioceptors), or altering the tone of the cord (electric, magnetic, and pharmacologic stimulation) appear to that each method has great promise. Lessons from noxious stimulation experiments demonstrating the ability of the cord to learn operant and classical conditioning, especially to noxious stimuli, should caution caregivers in therapeutic design as some schedules of learning are maladaptive and could result in inadvertent reductions in function. Rehabilitation of the spinal cord, like other regions of the CNS, will have to choose a stimulus or the stimuli that best serve the behavior to be restored, and do so in a schedule that matches the ability of the spinal cord to constructively integrate these inputs.

Acknowledgments This review was made possible by generous support from the J. Yang & Family Foundation. The research described was conducted in the UCLA Clinical and Translational Research Center (CTRC), which was supported by NIH/National Center for Advancing Translational Science (NCATS) UCLA CTSI Grant Number UL1TR000124. D.C.L. is a 1999 Paul & Daisy Soros New American Fellow.

Compliance with Ethics Guidelines

Conflict of Interest Tianyi Niu, William A. Alaynick, and Daniel C. Lu declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:

- Of importance

1. Alaynick WA, Jessell TM, Pfaff SL. SnapShot: spinal cord development. Cell. 2011;146:178e1.
2. • Lu DC, Niu T, Alaynick WA. Molecular and cellular development of spinal cord locomotor circuitry. Front Mol Neurosci. 2015;8. *This paper reviews several interneuron classes that contribute to the CPG in experimental animals.*
3. Brown TG. The intrinsic factors in the act of progression in the mammal. Proceedings of the Royal Society of London, Series B (containing papers of a biological character). 1911;19:308–19.
4. Grillner S. Neurobiological bases of rhythmic motor acts in vertebrates. Science. 1985;228:143–9.
5. Grillner S, Jessell TM. Measured motion: searching for simplicity in spinal locomotor networks. Curr Opin Neurobiol. 2009;19:572–86.
6. Goulding M. Circuits controlling vertebrate locomotion: moving in a new direction. Nat Rev Neurosci. 2009;10:507–18.
7. Rossignol S, Dubuc R, Gossard J-P. Dynamic sensorimotor interactions in locomotion. Physiol Rev. 2006;86:89–154.
8. Horak FB, Nashner LM. Central programming of postural movements: adaptation to altered support-surface configurations. J Neurophysiol. 1986;55:1369–81.
9. Schubert M, Curt A, Jensen L, Dietz V. Corticospinal input in human gait: modulation of magnetically evoked motor responses. Exp Brain Res. 1997;115:234–46.
10. Edgerton V, Roy R, Hodgson J, Prober R, De Guzman C, De Leon R. Potential of adult mammalian lumbosacral spinal cord to execute and acquire improved locomotion in the absence of supraspinal input. J Neurotrauma. 1992;9:S119–28.
11. Fukuyama H, Ouchi Y, Matsuzaki S, Nagahama Y, Yamauchi H, et al. Brain functional activity during gait in normal subjects: a SPECT study. Neurosci Lett. 1997;228:183–6.
12. Miyai I, Tanabe HC, Sase I, Eda H, Oda I, et al. Cortical mapping of gait in humans: a near-infrared spectroscopic topography study. Neuroimage. 2001;14:1186–92.
13. Suzuki M, Miyai I, Ono T, Oda I, Konishi I, et al. Prefrontal and premotor cortices are involved in adapting walking and running speed on the treadmill: an optical imaging study. Neuroimage. 2004;23:1020–6.
14. Cazalets JR, Bertrand S. Coupling between lumbar and sacral motor networks in the neonatal rat spinal cord. Eur J Neurosci. 2000;12:2993–3002.
15. Nathan P, Smith M, Deacon P. Vestibulospinal, reticulospinal and descending propriospinal nerve fibres in man. Brain. 1996; 119:1809–33.
16. Barbeau H, Rossignol S. Recovery of locomotion after chronic spinalization in the adult cat. Brain Res. 1987;412:84–95.
17. De Leon R, Hodgson J, Roy R, Edgerton V. Locomotor capacity attributable to step training versus spontaneous recovery after spinalization in adult cats. J Neurophysiol. 1998;79:1329–40.
18. Lovely R, Gregor R, Roy R, Edgerton V. Weight-bearing hindlimb stepping in treadmill-exercised adult spinal cats. Brain Res. 1990;514:206–18.
19. Harkema S, Gerasimenko Y, Hodes J, Burdick J, Angeli C, et al. Effect of epidural stimulation of the lumbosacral spinal cord on voluntary movement, standing, and assisted stepping after motor complete paraplegia: a case study. The Lancet. 2011;377:1938–47.
20. Grillner S. Locomotion in vertebrates: central mechanisms and reflex interaction. Physiol Rev. 1975;55:247–304.
21. Grillner S, Zangger P. On the central generation of locomotion in the low spinal cat. Exp Brain Res. 1979;34:241–61.
22. Groves PM, DeMarco R, Thompson RF. Habituation and sensitization of spinal interneuron activity in acute spinal cat. Brain Res. 1969;14:521–5.
23. Thompson RF, Spencer WA. Habituation: a model phenomenon for the study of neuronal substrates of behavior. Psychol Rev. 1966;73:16.
24. Buerger A, Fennessy A. Learning of leg position in chronic spinal rats. Nature. 1970;225:751–2.
25. Grau JW, Crown ED, Ferguson AR, Washburn SN, Hook MA, Miranda RC. Instrumental learning within the spinal cord: underlying mechanisms and implications for recovery after injury. Behav Cogn Neurosci Rev. 2006;5:191–239.
26. Sekhon LH, Fehlings MG. Epidemiology, demographics, and pathophysiology of acute spinal cord injury. Spine. 2001;26:S2–12.
27. Raineteau O, Schwab ME. Plasticity of motor systems after incomplete spinal cord injury. Nat Rev Neurosci. 2001;2: 263–73.
28. Ferguson AR, Huie JR, Crown ED, Grau JW. Central nociceptive sensitization vs. spinal cord training: opposing forms of plasticity that dictate function after complete spinal cord injury. Front Physiol. 2012;3:396.

29. Bareyre FM, Schwab ME. Inflammation, degeneration and regeneration in the injured spinal cord: insights from DNA microarrays. Trends Neurosci. 2003;26:555–63.
30. Kiehn O. Locomotor circuits in the mammalian spinal cord. Annu Rev Neurosci. 2006;29:279–306.
31. Ichiyama RM, Courtine G, Gerasimenko YP, Yang GJ, van den Brand R, et al. Step training reinforces specific spinal locomotor circuitry in adult spinal rats. J Neurosci. 2008;28:7370–5.
32. Giszter SF, Hockensmith G, Ramakrishnan A, Udoekwere UI. How spinalized rats can walk: biomechanics, cortex, and hindlimb muscle scaling–implications for rehabilitation. Ann N Y Acad Sci. 2010;1198:279–93.
33. Anderson KD, Gunawan A, Steward O. Quantitative assessment of forelimb motor function after cervical spinal cord injury in rats: relationship to the corticospinal tract. Exp Neurol. 2005;194:161–74.
34. Hiersemenzel LP, Curt A, Dietz V. From spinal shock to spasticity: neuronal adaptations to a spinal cord injury. Neurology. 2000;54:1574–82.
35. Dietz V, Harkema SJ. Locomotor activity in spinal cord-injured persons. J Appl Physiol. 2004;96:1954–60.
36. •• Hubli M, Bolliger M, Dietz V. Neuronal dysfunction in chronic spinal cord injury. Spinal Cord. 2011;49:582–7. *This paper summarized electrophysiologic changes in human subjects after chronic (1 year) spinal cord injury.*
37. Dietz V, Grillner S, Trepp A, Hubli M, Bolliger M. Changes in spinal reflex and locomotor activity after a complete spinal cord injury: a common mechanism? Brain. 2009;132:2196–205.
38. Adkins DL, Boychuk J, Remple MS, Kleim JA. Motor training induces experience-specific patterns of plasticity across motor cortex and spinal cord. J Appl Physiol. 2006;101:1776–82.
39. Goldshmit Y, Lythgo N, Galea MP, Turnley AM. Treadmill training after spinal cord hemisection in mice promotes axonal sprouting and synapse formation and improves motor recovery. J Neurotrauma. 2008;25:449–65.
40. Hutchinson KJ, Gomez-Pinilla F, Crowe MJ, Ying Z, Basso DM. Three exercise paradigms differentially improve sensory recovery after spinal cord contusion in rats. Brain. 2004;127:1403–14.
41. Liu M, Stevens-Lapsley JE, Jayaraman A, Ye F, Conover C, et al. Impact of treadmill locomotor training on skeletal muscle IGF1 and myogenic regulatory factors in spinal cord injured rats. Eur J Appl Physiol. 2010;109:709–20.
42. Winchester P, McColl R, Querry R, Foreman N, Mosby J, et al. Changes in supraspinal activation patterns following robotic locomotor therapy in motor-incomplete spinal cord injury. Neurorehabil Neural Repair. 2005;19:313–24.
43. Enzinger C, Dawes H, Johansen-Berg H, Wade D, Bogdanovic M, et al. Brain activity changes associated with treadmill training after stroke. Stroke. 2009;40:2460–7.
44. Eccles RM, Lundberg A. Significance of supraspinal control of reflex actions by impulses in muscle afferents. Experientia. 1958;14:197–9.
45. Perez MA, Field-Fote EC, Floeter MK. Patterned sensory stimulation induces plasticity in reciprocal ia inhibition in humans. J Neurosci. 2003;23:2014–8.
46. •• Mehrholz J, Kugler J, Pohl M. Locomotor training for walking after spinal cord injury. Spine. 2008;33:E768–77. *This is a Cochran review of the published RCTs regarding functional recoveries in SCI subjects with a variety of rehabilitation methods.*
47. Harkema SJ. Plasticity of interneuronal networks of the functionally isolated human spinal cord. Brain Res Rev. 2008;57:255–64.
48. Gurfinkel V, Levik YS, Kazennikov O, Selionov V. Locomotor-like movements evoked by leg muscle vibration in humans. Eur J Neurosci. 1998;10:1608–12.
49. Selionov VA, Ivanenko YP, Solopova IA, Gurfinkel VS. Tonic central and sensory stimuli facilitate involuntary air-stepping in humans. J Neurophysiol. 2009;101:2847–58.
50. Gerasimenko Y, Gorodnichev R, Machueva E, Pivovarova E, Semyenov D, et al. Novel and direct access to the human locomotor spinal circuitry. J Neurosci. 2010;30:3700–8.
51. Courtine G, Song B, Roy RR, Zhong H, Herrmann JE, et al. Recovery of supraspinal control of stepping via indirect propriospinal relay connections after spinal cord injury. Nat Med. 2008;14:69–74.
52. Courtine G, Gerasimenko Y, van den Brand R, Yew A, Musienko P, et al. Transformation of nonfunctional spinal circuits into functional states after the loss of brain input. Nat Neurosci. 2009;12:1333–42.
53. Lapointe NP, Guertin PA. Synergistic effects of D1/5 and 5-HT1A/7 receptor agonists on locomotor movement induction in complete spinal cord-transected mice. J Neurophysiol. 2008;100:160–8.
54. Rossignol S, Giroux N, Chau C, Marcoux J, Brustein E, Reader T. Pharmacological aids to locomotor training after spinal injury in the cat. J Physiol. 2001;533:65–74.
55. Domingo A, Al-Yahya AA, Asiri Y, Eng JJ, Lam, Spinal Cord Injury Rehabilitation Evidence Research Team T. A systematic review of the effects of pharmacological agents on walking function in people with spinal cord injury. J Neurotrauma. 2012;29:865–79.
56. Cogiamanian F, Vergari M, Pulecchi F, Marceglia S, Priori A. Effect of spinal transcutaneous direct current stimulation on somatosensory evoked potentials in humans. Clin Neurophysiol. 2008;119:2636–40.
57. Cortes M, Thickbroom GW, Valls-Sole J, Pascual-Leone A, Edwards DJ. Spinal associative stimulation: a non-invasive stimulation paradigm to modulate spinal excitability. Clin Neurophysiol. 2011;122:2254–9.
58. Norton JA, Mushahwar VK. Afferent inputs to mid- and lower-lumbar spinal segments are necessary for stepping in spinal cats. Ann N Y Acad Sci. 2010;1198:10–20.
59. • Knikou M. Functional reorganization of soleus H-reflex modulation during stepping after robotic-assisted step training in people with complete and incomplete spinal cord injury. Exp Brain Res. 2013;228:279–96. *This paper demonstrates that the human spinal cord can learn from proprioceptive inputs that are generated during treadmill stepping.*
60. Fallon JB, Macefield VG. Vibration sensitivity of human muscle spindles and Golgi tendon organs. Muscle Nerve. 2007;36:21–9.
61. Askari S, Chao T, Conn L, Partida E, Lazzaretto T, et al. Effect of functional electrical stimulation (FES) combined with robotically assisted treadmill training on the EMG profile. In: Proceedings of the annual international conference of the IEEE Engineering in Medicine and Biology Society. IEEE; 2011. p. 3043–46.
62. Gorodnichev RM, Pivovarova EA, Pukhov A, Moiseev SA, Savokhin AA, et al. Transcutaneous electrical stimulation of the spinal cord: non-invasive tool for activation of locomotor circuitry in human. Fiziol Cheloveka. 2012;38:46–56.
63. • Duclos C, Kemlin C, Lazert D, Gagnon D, Dyer JO, Forget R. Complex muscle vibration patterns to induce gait-like lower-limb movements: proof of concept. J Rehabil Res Dev. 2014;51:245–51. *Use of vibratory stimulation to recapitualte proprioceptive sensory information and activate CPG circuitry.*
64. AuYong N, Lu DC. Neuromodulation of the lumbar spinal locomotor circuit. Neurosurg Clin N Am. 2014;25:15–23.
65. Edgerton VR, Harkema S. Epidural stimulation of the spinal cord in spinal cord injury: current status and future challenges. Expert Rev Neurother. 2011;11:1351–3.

66. • Gerasimenko Y, Gorodnichev RM, Pukhov A, Moshonkina TR, Savochin A, et al. Initiation and modulation of locomotor circuitry output with multi-site transcutaneous electrical stimulation of the spinal cord in non-injured humans. J Neurophysiol. 2014;113:837–42. *This paper demosntrates the use of transcutaneous stimulation using the "Russian Stimulation" pattern of a 10 kHz carrier wave turned on for several millisconds at 10–40 times per socnond. This method appears to activate spinal CPG circuitry in intact individuals.*
67. Adams MM, Ditor DS, Tarnopolsky MA, Phillips SM, McCartney N, Hicks AL. The effect of body weight-supported treadmill training on muscle morphology in an individual with chronic, motor-complete spinal cord injury: a case study. J Spinal Cord Med. 2006;29:167–71.
68. Forrest GF, Sisto SA, Barbeau H, Kirshblum SC, Wilen J, et al. Neuromotor and musculoskeletal responses to locomotor training for an individual with chronic motor complete AIS-B spinal cord injury. J Spinal Cord Med. 2008;31:509–21.
69. Ditor DS, Kamath MV, MacDonald MJ, Bugaresti J, McCartney N, Hicks AL. Effects of body weight-supported treadmill training on heart rate variability and blood pressure variability in individuals with spinal cord injury. J Appl Physiol. 2005;98:1519–25.
70. Soyupek F, Savas S, Ozturk O, Ilgun E, Bircan A, Akkaya A. Effects of body weight supported treadmill training on cardiac and pulmonary functions in the patients with incomplete spinal cord injury. J Back Musculoskeletal Rehabil. 2009;22:213–8.
71. Turiel M, Sitia S, Cicala S, Magagnin V, Bo I, et al. Robotic treadmill training improves cardiovascular function in spinal cord injury patients. Int J Cardiol. 2011;149:323–9.
72. Hesse S, Werner C, Bardeleben A. Electromechanical gait training with functional electrical stimulation: case studies in spinal cord injury. Spinal Cord. 2004;42:346–52.
73. Manella KJ, Torres J, Field-Fote EC. Restoration of walking function in an individual with chronic complete (AIS A) spinal cord injury. J Rehabil Med. 2010;42:795–8.